2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of zinc. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

Zinc is an essential nutrient in humans and animals that is necessary for the function of a large number of metalloenzymes. These enzymes include alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, super-oxide dismutase, and deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerase. As such, zinc is required for normal nucleic acid, protein, and membrane metabolism, as well as cell growth and division. Zinc also plays an essential role in the maintenance of nucleic acid structure of genes (zinc finger phenomenon). Zinc deficiency has been associated with dermatitis, anorexia, growth retardation, poor wound healing, hypogonadism with impaired reproductive capacity, impaired immune function, and depressed mental function; an increased incidence of congenital malformations in infants has also been associated with zinc deficiency in the mothers (Cotran et al. 1989; Elinder 1986; Sandstead 1981). Therefore, certain levels of zinc intake are recommended. The RDA for zinc is 15 mg/day in men and 12 mg/day in women (NAS/NRC 1989b). Higher RDAs are recommended for women during pregnancy and lactation (15 mg/day for pregnant women, 19 mg/day for nursing women during the first 6 months, and 16 mg/day during the second 6 months of nursing).

Just as zinc deficiency has been associated with adverse effects in humans and animals, overexposures to zinc also have been associated with toxic effects. This chapter contains a description of the toxic effects that have been associated with exposures to high levels of zinc and selected zinc compounds by the inhalation, oral, and dermal routes. Specifically, zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide will be considered. Other zinc compounds are discussed in this chapter whenever data regarding these compounds add relevant information to the

discussion on zinc. Any general comments regarding the lack of data on zinc refer to both zinc and its compounds.

Because there are differences in toxicity between the various zinc compounds following inhalation exposure, these compounds will be discussed under separate subheadings in Section 2.2.1 (Inhalation Exposure). After oral or dermal exposure, the toxicities are comparable for all zinc compounds. Therefore, in Section 2.2.2 (Oral Exposure) and Section 2.23 (Dermal Exposure), the discussion will not be subdivided, but the specific zinc compounds will be identified in each case.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure - inhalation, oral, and dermal; and then by health effect - death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods - acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure (LSE) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects

is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the LSE tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

2.2.1 Inhalation Exposure

2.2.1.l Death

In humans, death has resulted from acute exposure to zinc compounds. When a high concentration (estimated at 33,000 mg zinc/m^3) of zinc chloride smoke resulted from the explosion of many generators in a tunnel following a bombing raid in World War II, 10 of the 70 exposed people in the tunnel died within 4 days (Evans 1945). The smoke generated contained mainly zinc chloride, but exposure to other constituents, namely zinc oxide, hexachloroethane, calcium silicate, and an igniter, was also possible. Therefore, the deaths resulting from the explosion cannot be conclusively attributed to exposure to zinc chloride only. This is the only human study reporting an estimated exposure level that caused death. Hence, this level is reported as a LOAEL in Table 2-l and Figure 2-1. Another study reported the death of a fireman exposed to the contents of a smoke bomb in a closed environment (Milliken et al. 1963). The man died 18 days after exposure because of respiratory difficulty. Again, exposure to zinc chloride was simultaneous with exposure to other substances in the smoke. Two soldiers exposed without gas masks to zinc chloride smoke during military training developed severe adult respiratory distress syndrome (ARDS) and died 25-32 days after the incident (Hjortso et al. 1988). Diffuse microvascular obliteration, widespread occlusion of the pulmonary arteries, and extensive interstitial and intra-alveolar fibrosis were observed at autopsy. Zinc levels in major

TABLE 2-1. Levels of Significant Exposure to Zinc - Inhalation

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL ($mg\ Zn/m^3$)	LOAEL (effect) Less serious ($mg\ Zn/m^3$)	LOAEL (effect) Serious ($mg\ Zn/m^3$)	Reference	Form
ACUTE EXPOSURE								
Death								
1	Human	<1 hr				33000 (10/70 died)	Evans 1945	chloride
Systemic								
2	Human	2 hr	Resp	.0036			Linn et al. 1981	amm sulfate
3	Human	1 d 2hr/d	Resp		3.9 (dyspnea, increased airway resistance)		Gordon et al. 1992	oxide
			Other		3.9 (fever/chills)			
4	Human	15-30 min	Resp		77 (minimal change in pulmonary function)		Blanc et al. 1991	oxide
5	Human	6-8 hr (occup)	Resp	0.034			Marquart et al. 1989	oxide
6	Human	10.5-12 min	Resp		600 (decreased vital capacity)		Sturgis et al. 1927	oxide
			Gastro		600 (nausea)			
			Hemato		600 (increased leukocytes)			
7	Rat	1 d 3hr/d	Resp		2.2 (increased LDH protein in broncho-alveolar lavage fluid)		Gordon et al. 1992	oxide
8	Rabbit	1 d 2hr/d	Resp	4.6			Gordon et al. 1992	oxide
9	Gn pig	1-3 d 3hr/d	Resp	1.8	4.7 (increased neutrophils, LDH, and protein in broncho-alveolar lavage fluid)		Conner et al. 1988	oxide

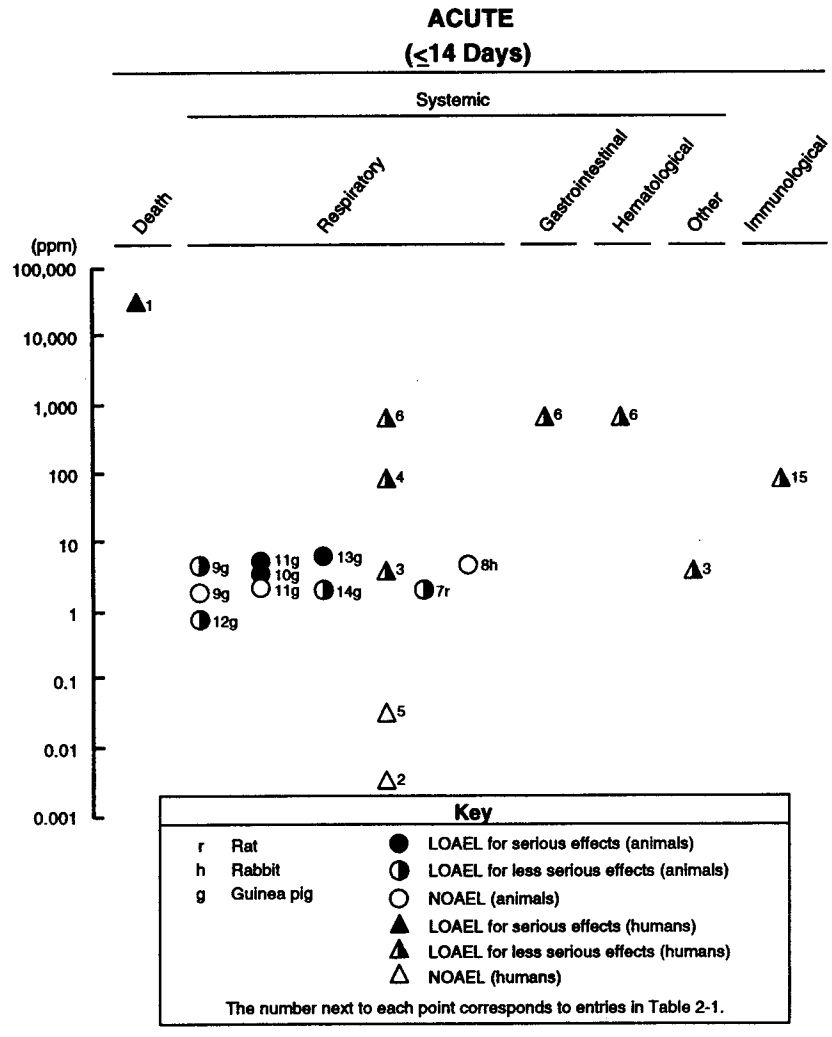
TABLE 2-1. Levels of Significant Exposure to Zinc - Inhalation (continued)

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL ($mg\ Zn/m^3$)	LOAEL (effect) Less serious ($mg\ Zn/m^3$)	LOAEL (effect) Serious ($mg\ Zn/m^3$)	Reference	Form
10	Gn pig	6 d 3hr/d	Resp			3.7 (impaired lung function; inflammation; increased pulmonary resistance; increased lung weight)	Lam et al. 1985	oxide
11	Gn pig	5 d 3hr/d	Resp	2.2		5.6 (impaired lung function; increased lung weight)	Lam et al. 1988	oxide
12	Gn pig	1 hr	Resp		0.73 (decreased compliance)		Amdur et al. 1982	oxide
13	Gn pig	3 hr	Resp			6.3 (decreased functional residual capacity)	Lam et al. 1982	oxide
14	Gn pig	1 d 3hr/d	Resp		2.2 (increased LDH and protein in bronchoalveolar lavage fluid)		Gordon et al. 1992	oxide
Immunological								
15	Human	15-30 min			77 (increased number of leukocytes, T cells, T suppressor cells, and natural killer cells in bronchoalveolar lavage fluid)		Blanc et al. 1991	oxide

[a]The number corresponds to entries in Figure 2-1.

amm sulfate = ammonium sulfate; chloride = zinc chloride; d = day(s); Gastro = gastrointestinal; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; (occup) = occupational; oxide = zinc oxide; Resp = respiratory; Zn = zinc

FIGURE 2-1. Levels of Significant Exposure to Zinc - Inhalation



organs and tissues obtained during autopsy were within the normal range, and no zinc particles were observed by scanning electron microscopy. According to the authors, the fumes from the smoke bombs consisted mainly of zinc chloride. However, no exposure levels were estimated, and other substances were also present in the smoke.

A case study presented by Murray (1926) reported on an infant death due to bronchopneumonia resulting from inhalation, and possibly ingestion of an unspecified amount of zinc stearate powder spilled from a container. However, it is unclear whether the death was due to the zinc content or whether aspiration bronchopneumonia would result from inhalation of similar powders that do not contain zinc.

In mice, the reported LCT_{50}, (product of lethal concentration and time to kill 50% of animals) of zinc chloride is 11,800 mg-min/m^3 (Schenker et al. 1981). However, Schenker et al. (1981) did not provide information on how this value was determined. Following exposure to zinc chloride smoke for 3-20 weeks, mortality was 50% in mice exposed to 121.7 mg zinc/m^3 (compared to 20% in controls) and 22% in guinea pigs exposed to 119.3 mg zinc/m^3 (compared to 8% in controls) (Marrs et al. 1988). The smoke was similar to that described by Evans (1945) and also contained zinc oxide, hexachloroethane, and other compounds.

2.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal effects in humans or animals after inhalation exposure to zinc or zinc compounds. The systemic effects observed after inhalation exposure are discussed below. In most cases, the effects of zinc are discussed without separating effects caused by the individual zinc compounds. However, the respiratory effects of the individual zinc compounds are discussed separately because the nature of the respiratory toxicity differs depending on the particular compound to which one is exposed. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects

Zinc Oxide. Metal fume fever, a well-documented acute disease induced by intense inhalation of metal oxides, especially zinc, temporarily impairs pulmonary function but does not progress to chronic lung disease (Brown 1988; Drinker et al. 1927b; Malo et al. 1990). Symptoms generally appear within a few hours after acute exposure, usually with dryness of the throat and coughing (Drinker and Drinker 1927b). The most prominent respiratory effects of metal fume fever are substernal chest pain, cough, and dyspnea (Rohrs 1957). The impairment of pulmonary function is characterized by reduced lung volumes and a decreased diffusing capacity of carbon monoxide (Malo et al. 1990; Vogelmeier et al. 1987). The respiratory effects have been shown to be accompanied by an increase in bronchiolar leukocytes (Vogelmeier et al. 1987). The respiratory symptoms generally disappear in the exposed individual within l-4 days (Brown 1988; Drinker et al. 1927b; Sturgis et al. 1927). Refer to the Other Systemic Effects section below for further details on nonrespiratory effects related to metal fume fever. Inhalation of zinc oxide is most likely to occur in occupational situations where zinc smelting or welding take place. Ultrafine zinc oxide particles (0.2-1.0 pm) originate from heating zinc beyond its boiling point in an oxidizing atmosphere. Upon inhalation, these small particles (< 1 pm) reach the alveoli and cause inflammation and tissue damage in the lung periphery (Brown 1988; Drinker et al. 1927b; Vogelmeier et al. 1987).

A number of studies have measured exposure levels associated with metal fume fever. Workers involved in pouring molten zinc reported shortness of breath and chest pains 2-12 hours following exposure to 320-580 mg zinc/m^3 as zinc oxide for l-3 hours (Hammond 1944); the number of workers was not reported. Two volunteers had nasal passage irritation, cough, substernal chest pain, persistent rales of the lung base, and a decreased vital capacity for approximately 3-49 hours following acute inhalation (10-12 minutes) of 600 mg zinc/m^3 as zinc oxide (Sturgis et al. 1927). A subject experimentally exposed to zinc oxide fumes reported mild pain when breathing deeply the next day after a 5-hour exposure to 430 mg zinc/m^3 (Drinker et al. 1927a). Minimal changes in forced expiratory flow were observed 1 hour after a 15-30-minute exposure to 77 mg zinc/m3 as zinc oxide (Blanc et al. 1991).

Acute experimental exposures to lower concentrations of zinc oxide (14 mg/m^3 for 8 hours or 45 mg zinc/m^3 for 20 minutes) and occupational exposures to similar concentrations (8-12 mg

zinc/m^3 for 1-3 hours and 0.034 mg zinc/m^3 for 6-8 hours) have not produced symptoms of metal fume fever (Drinker et al. 1927b; Hammond 1944; Marquart et al. 1989). In a single-blind experiment, exposure of subjects to 3.9 mg zinc/m3 as zinc oxide resulted in sore throat and chest tightness but no impairment of pulmonary function (Gordon et al. 1992). It is speculated that subjects in other studies may have been less susceptible because of the development of tolerance to zinc (Gordon et al. 1992). Recurrent episodes of cough and dyspnea were reported in a former mild smoker 3 years after beginning work in a metal foundry where exposure to zinc oxide presumably occurred (Ameille et al. 1992). This case was distinguishable from metal fume fever because of the lack of tolerance to zinc (as shown by the late emergence of symptoms).

Several animal studies have been conducted to quantitate specific effects after acute zinc oxide inhalation. As in human exposure, the respiratory system is the primary site of injury following inhalation exposure. Acute administration of 88-482 mg zinc/m^3 as zinc oxide to rats and rabbits resulted in the following pulmonary changes: grayish areas with pulmonaty congestion, various degrees of peribronchial leukocytic infiltration, and exudate composed almost entirely of polymorphonuclear leukocytes in bronchi (Drinker and Drinker 1928). A minimum effect level could not be determined because the concentration varied widely during exposure. Cats similarly exposed exhibited more severe effects including bronchopneumonia, leukocyte infiltration into alveoli, and grayish areas with congestion. During the exposure period, the cats demonstrated labored breathing and evidence of upper respiratory tract obstruction.

Guinea pigs administered 0.73 mg zinc/m^3 as zinc oxide for 1 hour had a progressive decrease in lung compliance but no change in air flow resistance. These observations reflect a response in the lung periphery where submicrometer aerosols are likely to deposit (Amdur et al. 1982). The authors postulated that reduced compliance may be associated with human metal fume fever.

In contrast to the results of Amdur et al. (1982), no effects on ventilation, lung mechanics (respiratory frequency, tidal volume, pulmonary resistance, and pulmonary compliance), diffusing capacity of carbon monoxide, or most lung volume parameters were observed by Lam et al. (1982) following the exposure of guinea pigs to 6.3 mg zinc/m^3 as zinc oxide for 3 hours. However, functional residual capacity was significantly decreased. The discrepancy between the results of Amdur et al. (1982) and Lam et al. (1982) may be attributable to the use of anesthetized animals by Lam et al. (1982). In a later study, exposures of guinea pigs to 3.7 or 4.3 mg zinc/m^3 as zinc

oxide for 3 hours/day, for 6 days, resulted in transient functional, morphological, and biochemical changes (Lam et al. 1985). Functional changes included increased flow resistance, decreased lung compliance, and decreased diffusing capacity, all of which returned to normal within 24-72 hours following exposure. The morphological changes (increased lung weight, inflammation involving the proximal portion of alveolar ducts and adjacent alveoli, interstitial thickening, and increased pulmonary macrophages and neutrophils in adjacent air spaces) were, however, still present at 72 hours. In guinea pigs with evidence of an inflammatory reaction involving the peripheral airways, DNA synthesis increased in bronchiolar cells. Similarly, exposure of guinea pigs to 5.6 mg zinc/m^3 as zinc oxide for 3 hours/day, for 5 days, resulted in gradual decreases in total lung capacity, vital capacity, and decreased carbon monoxide diffusing capacity (Lam et al. 1988); however, no effects were observed in guinea pigs exposed to 2.2 mg zinc/m^3. The reason that effects have been seen in the guinea pig at exposure levels lower than humans may have to do with the structural features of the guinea pig lung. The bronchi and peripheral airways of guinea pigs have a thicker smooth muscle layer and only a small surface area covered by alveolar sacs compared to the bronchi and peripheral airways of other laboratory animals and humans. This makes the guinea pig more susceptible than other laboratory animals to functional impairment of the peripheral airways and should be noted in toxicity comparisons (Lam et al. 1982).

The bronchoalveolar lavage fluid of rats or guinea pigs exposed to 2.2 mg zinc/m^3 for 3 hours contained increased levels of lactate dehydrogenase and protein, suggesting effects on cell viability or membrane permeability (Gordon et al. 1992). Rabbits were not affected following a similar exposure to 4.6 mg zinc/m^3 for 2 hours. Guinea pigs had foci of inflammation after exposure to 4.7 mg zinc/m^3 for 3 days, and the bronchoalveolar lavage fluid contained increased levels of protein, angiotensin converting enzyme, and neutrophils (Conner et al. 1988).

Zinc Chloride. Zinc chloride, a corrosive inorganic salt, is more damaging than zinc oxide to the mucous membranes of the nasopharynx and respiratory tract upon contact. Zinc chloride is a primary ingredient in smoke bombs used by the military for screening purposes, crowd dispersal, and occasionally in military and civilian fire-fighting exercises. Reports of serious respiratory injury have been reported to result from accidental inhalation of smoke from these bombs. These reports are of limited use in assessing the toxicity of zinc chloride because exposure to other compounds, usually hexachloroethane, zinc oxide, and calcium silicides, also occur. Furthermore, the specific concentrations inhaled are usually unknown. Despite these limitations, several case

studies have described similar respiratory effects in humans following acute inhalation exposures. These effects include dyspnea, cough, pleuritic chest pain, bilateral diffuse infiltrations, pneumothorax, and acute pneumonitis from respiratory tract irritation (Johnson and Stonehill 1961; Matarese and Matthews 1966; Schenker et al. 1981). In the study by Johnson and Stonehill (1961) cough, dyspnea, burning throat, diffuse infiltrates throughout the lung, chemical pneumonitis, and decreased vital capacity were observed at an estimated zinc chloride exposure level of 4,075 mg/m^3 (1,955 mg zinc/m^3). In other studies, more severe effects have occurred, including ulcerative and edematous changes in mucous membranes, fibrosis, subpleural hemorrhage, advanced pulmonary fibrosis, and fatal respiratory distress syndrome (Evans 1945; Hjortso et al. 1988; Homma et al. 1992; Milliken et al. 1963).

Focal alveolitis, consolidation, emphysema, infiltration with macrophages, and fibrosis were observed in guinea pigs that died following exposure to 119 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for up to 3 weeks (Marrs et al. 1988). Thirteen months after a 20-week exposure, rats and mice inhaling 121.7 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, showed increased macrophages in the lungs (Marrs et al. 1988). The smoke also contained zinc oxide, hexachloroethane, and other compounds.

Zinc Ammonium Sulfate. Zinc ammonium sulfate is a compound emitted during combustion of fossil fuels and is, therefore, found in the ambient air. Humans acutely exposed to a concentration of 0.0036 mg zinc/m^3 as zinc ammonium sulfate for 2 hours exhibited minimal or no short-term respiratory effects (Linn et al. 1981). However, most human exposures to an ambient air pollutant such as zinc ammonium sulfate are chronic, and this study provides little information about the health effects associated with typical exposures.

No studies were located regarding respiratory effects in animals after inhalation exposure to zinc ammonium sulfate.

Zinc Stearute. Inhalation of zinc stearate powder resulted in aspiration bronchopneumonia in an infant (Murray 1926). However, it is unclear whether the bronchopneumonia resulted from the inhalation of zinc stearate powder specifically or from the inhalation of powders in general.

2. HEALTH EFFECTS

No studies were located regarding respiratory effects in animals after inhalation exposure to zinc stearate.

Cardiovascular Effects. No atypical heart sounds or blood pressure abnormalities were observed in 24 employees occupationally exposed to concentrations as high as 130 mg zinc/m^3 of metallic zinc dust, zinc oxide dust, zinc sulfide dust, or lithophone dust (a combination of barium sulphate and ≈30% zinc sulphide) for 2-35.5 years (Batchelor et al. 1926). However, this study is limited because only selected employees were examined, and they were not compared to controls.

Extremely limited information was located regarding cardiovascular effects in animals following inhalation exposure to zinc. Routine gross and microscopic examination of the hearts of rats and mice revealed no adverse effects 13 months after exposure to 121.7 mg zinc/m^3 as zinc chloride smoke (also containing other compounds) for 1 hour/day, 5 days/week, for 20 weeks (Marrs et al. 1988). Similarly, no changes were observed in the hearts of guinea pigs exposed to 119.3 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 3 weeks, and then observed for an additional 17 months (Marrs et al. 1988).

Gastrointestinal Effects. Nausea was reported by humans exposed to high concentrations of zinc oxide fumes (Hammond 1944; Rohrs 1957; Sturgis et al. 1927) and zinc chloride smoke (Evans 1945; Johnson and Stonehill 1961; Schenker et al. 1981). The zinc chloride smoke also contained zinc oxide, hexachloroethane, and other compounds. In general, exposure levels associated with nausea have not been reported. However, exposures to 320 mg zinc/m^3 as zinc oxide for l-3 hours (Hammond 1944) or 600 mg zinc/m^3 as zinc oxide for lo-12 minutes (Sturgis et al. 1927) were both reported to have resulted in nausea. Autopsies of victims who died following exposure to very high concentrations of zinc chloride smoke revealed irritation of the stomach and intestines (Evans 1945). The smoke also contained zinc oxide, hexachloroethane, and other compounds. Workers in the galvanizing industry were found by McCord et al. (1926) to have a higher than expected incidence of gastrointestinal problems; however, these individuals may have been exposed to other chemicals (arsenic, hydrogen sulfide). Of 15 workers examined with 7-20 years of experience, 12 had frequent episodes of epigastric or abdominal pain, nausea, vomiting, ulcers, constipation, tarry stools, and/or gas. It is unclear whether these effects were due to systemic zinc or were the result of direct contact with the gastrointestinal tract following mucociliary clearance of inhaled zinc particles and subsequent swallowing. In contrast, 24 workers

with 2-35.5 years of exposure to ≤130 mg zinc/m^3 as metallic zinc dust, zinc sulfide dust, zinc oxide, or lithophone dust reported no nausea or vomiting and only occasional mild abdominal discomfort that could not be attributed with certainty to zinc exposure (Batchelor et al. 1926). A study examining the acidity of the stomach contents after stimulation in controls and workers employed in the production of brass alloys showed that stomach acidity was similar in the two groups prior to stimulation but remained elevated for longer periods after stimulation in the exposed workers (Hamdi 1969). This was proposed to account for the gastric complaints of workers exposed to zinc fumes. Despite these findings, X-rays showed no lesions in the stomachs or duodenums of exposed workers.

The only information available regarding gastrointestinal effects in animals was found in a study by Marrs et al. (1988) in which rats and mice were exposed to 121.7 mg zinc/m^3 as zinc chloride smoke (which also contains zinc oxide, hexachlorophene, and other compounds) for 1 hour/day, 5 days/week, for 20 weeks, and then observed for an additional 13 months. In the same study, guinea pigs were exposed to 119.3 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 3 weeks. All animals were sacrificed at the end of 18 months. Routine gross and microscopic evaluation of the stomach and intestines at 18 months revealed no persistent adverse effects.

Hematological Effects. Leukocytosis persisting for approximately 12 hours after fever dissipates is one of the hallmarks of metal fume fever (Mueller and Seger 1985). Such effects have been observed in a number of case reports of occupational and experimental exposure of humans to zinc oxide fumes (Brown 1988; Drinker et al. 1927a; Malo et al. 1990; Rohrs 1957; Sturgis et al. 1927). Increased leukocyte counts were observed following experimental exposures to 430 mg zinc/m^3 as zinc oxide for 3 hours (Drinker et al. 1927a) or 600 mg zinc/m^3 as zinc oxide for lo-12 minutes (Sturgis et al. 1927). These studies are limited in that they used an inadequate number of subjects, lacked controls, and used impure zinc oxide.

Decreased numbers of red blood cells and hemoglobin were found in several workers with 7-20 years of experience in the galvanizing industry (McCord et al. 1926). However, there were excess tobacco use and alcohol consumption by workers and possible concurrent exposure to other chemicals (chloride, sulfide) which limit the study results. No anemia was detected among 12 workers exposed for 4-21 years to zinc oxide fumes in the production of brass alloys (Hamdi 1969). These workers may have also been exposed to magnesium, copper, and aluminum.

No studies were located regarding hematological effects in animals after inhalation exposure to zinc.

Hepatic Effects. Routine blood chemistries and examinations revealed no liver disease among 12 workers with 4-21 years of exposure to zinc oxide fumes in the production of brass alloys (Hamdi 1969).

No adverse effects were observed during gross and microscopic examination of livers of rats and guinea pigs exposed to 121.7 mg zinc/m^3 or 119.3 mg zinc/m^3, respectively, as zinc chloride smoke for 1 hour/day, 5 days/week, for 20 weeks, and then sacrificed at the end of 18 months (Marrs et al. 1988). Significant increases in the incidence of fatty liver were observed in mice exposed to 12.8 or 121.7 mg zinc/m^3 as zinc chloride smoke using the same exposure paradigm; however, the incidence did not increase with dose (Marrs et al. 1988). The smoke contained other compounds in addition to zinc chloride.

Renal Effects. Urinalyses and histories of urinary function revealed no adverse effects in 24 workers exposed for 2-35.5 years to 5130 mg zinc/m^3 as metallic zinc dust, zinc sulfide dust, zinc oxide, or lithophone dust (Batchelor et al. 1926).

No adverse effects were observed following gross and microscopic examination of kidneys from rats, mice, and guinea pigs exposed for 1 hour/day, 5 days/week, for 20 weeks, to concentrations as high as 121.7 or 119.3 mg zinc/m^3 as zinc chloride smoke (which also contained other compounds) and then sacrificed 13 months later (Marrs et al. 1988).

Dermal/Ocular Effects. Reddened conjunctiva and cornea1 burns occurred in individuals exposed to high concentrations of zinc chloride smoke (estimated at 33,000 mg zinc/m^3) when several smoke generators exploded in a tunnel during World War II (Evans 1945). The ocular effects may have been due to direct contact with the smoke.

Other Systemic Effects. A fever appearing 3-10 hours after exposure to zinc oxide fumes and lasting approximately 24-48 hours is characteristic of metal fume fever caused by zinc (Mueller and Seger 1985). Elevated body temperature has been observed in a number of experimental and occupational zinc oxide exposures (Brown 1988; Drinker et al. 1927a; Hammond 1944; Malo et al.

1990; Rohrs 1957; Sturgis et al. 1927; Vogelmeier et al. 1987). Using a number of exposure concentrations for various durations, Drinker et al. (1927b) found that the increase in body temperature was dependent on the exposure duration and concentration. Based on their data, they calculated that the threshold for pyrogenic effects was 45 mg zinc/m^3 for 20 minutes. This study is limited in that impurities were present in the zinc used and no statistical analysis was performed. Exposure to zinc chloride smoke (which also contains other compounds) has also been associated with fever (Hjortso et al. 1988; Matarese and Matthews 1966).

No studies were located regarding other systemic effects in animals following inhalation exposure to zinc.

2.2.1.3 Immunological Effects

One report described hives and angioedema in a man exposed to zinc fumes at a zinc smelting plant (Farrell 1987). The author suggested that the patient had an immediate or delayed immunoglobulin E (IgE) response (or both) after a low dose of zinc fumes. Metal fume fever also resulted when the exposure increased. The signs and symptoms of toxicity were repeated in a challenge test conducted at the patient's home.

In a group of 14 welders acutely exposed to 77-153 mg zinc/m^3 as zinc oxide, significant correlations between the concentration of airborne zinc and the proportion of activated T cells, T helper cells, T inducer cells, T suppressor cells, and activated killer T cells were observed 20 hours after exposure (Blanc et al. 1991). In addition, significant increases in levels of polymorphonuclear leukocytes, macrophages, and all types of lymphocytes were observed in the bronchoalveolar lavage fluid 20 hours after exposure. Increased levels of lymphocytes, with a predominance of CD8 cells, in the bronchoalveolar lavage fluid were reported in a case study of a smelter exposed to unspecified levels of zinc fumes (Ameille et al. 1992).

The bronchoalveolar lavage fluid of rats or guinea pigs exposed to 2.2 mg zinc/m^3 for 3 hours contained increased levels of β-glucuronidase, suggesting a change in macrophage function (Gordon et al. 1992). Rabbits were not affected following a similar exposure to 4.6 mg zinc/m^3 for 2 hours. Rats, mice, and guinea pigs were exposed to concentrations as high as 119.3 or 121.7 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 20 weeks (Marrs et al.

1988). Routine gross and histopathologic examination of the lymph nodes, thymus, and spleen at the end of 18 months revealed no adverse effects. The smoke also contained zinc oxide, hexachlorophene, and other compounds.

2.2.1.4 Neurological Effects

Humans have reported nonspecific neurological effects such as headaches and malaise in association with other symptoms following inhalation of zinc oxide and in metal fume fever (Rohrs 1957; Sturgis et al. 1927). Staggering gait, hallucinations, and hilarity were observed in an individual who intentionally inhaled aerosols of metallic paint containing copper and zinc (Wilde 1975). However, it is most likely that these effects were due to exposure to hydrocarbon propellant rather than zinc.

No studies were located regarding neurological effects in animals after inhalation exposure to zinc.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to zinc.

Following an initial exposure of rats, mice, and guinea pigs to concentrations as high as 119.3 or 121.7 mg zinc/m^3 as zinc chloride smoke (which also contained other compounds) for 1 hour/day, 5 days/week, for 20 weeks, no adverse effects on the mammary glands, ovaries, fallopian tubes, or uteri were observed at 18 months (Marrs et al. 1988).

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to zinc.

2.2.1.7 Genotoxic Effects

Chromosome aberrations were observed in the lymphocytes of 24 workers in a zinc smelting plant (Bauchinger et al. 1976). However, the workers had increased blood levels of lead and cadmium, and the clastogenic effect was attributed to cadmium exposure.

Mice exposed by inhalation to zinc oxide had an increase in chromosomal aberrations in bone marrow cells (Voroshilin et al. 1978).

Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

In two epidemiological studies, workers did not have an increased incidence of cancers associated with occupational exposure (primarily inhalation exposure) to zinc (Logue et al. 1982; Neuberger and Hollowell 1982).

Workers in nine electrolytic zinc and copper refining plants were studied by Logue et al. (1982). The workers at two of these plants were exposed to zinc or zinc and copper; the other workers were exposed to copper. An association between cancer mortality and zinc exposure was not found.

Excess lung cancer mortality associated with residence in an old lead/zinc mining and smelting area of the midwestern United States was studied by Neuberger and Hollowell (1982). The ageand sex-adjusted mortality rates were compared to state and national rates. The analysis determined that lung cancer mortality was elevated in the region but was not found to be associated with exposure to environmental levels of lead or zinc. Many confounding factors were not considered in the analysis, such as smoking, occupation, and duration of residence in the area in question.

Female Porton strain mice (98-l00/group) exposed to 121.7 mg zinc/m^3 of a zinc oxide/ hexachloroethane smoke mixture (which produces zinc chloride), 1 hour/day, 5 days/week, for 20 weeks had a statistically significant increase in the incidence of alveologenic carcinoma (30%

versus 8% in control) thirteen months after the end of exposure (Marrs et al. 1988). No increased tumor incidences were seen in mice exposed to 1, 1.3, or 12.8 mg zinc/m^3. Guinea pigs and rats were also tested with similar dose levels, and no significant carcinogenic response was observed. A number of factors limits the usefulness of this study, including the presence of several compounds in the smoke that may have carcinogenic potential, the use of only female animals, and the short duration of the exposure (20 weeks).

2.2.2 Oral Exposure

Zinc has been orally administered in a variety of forms, such as zinc chloride, zinc sulfate, zinc oxide, powdered zinc, and others. Some of these compounds, such as zinc sulfate, have been administered in both hydrated and anhydrous forms. Study authors often do not state definitely which form was used in a particular study. Knowledge of the form used and its molecular weight is necessary to calculate the amount of elemental zinc administered under a given set of circumstances. If adequate information was not reported by the study authors, it was assumed that an anhydrous compound was used.

2.2.2.1 Death

In a case report presented by Murray (1926), an infant died from bronchopneumonia resulting from inhalation and ingestion of an unspecified amount of zinc stearate powder spilled from a container. However, the cause of death (bronchopneumonia) suggests that it resulted from the inhalation exposure, rather than the oral exposure, and it is unclear whether the lung damage resulted from the inhalation of zinc stearate powder specifically or from the inhalation of powders in general.

The LD_{50} values of several zinc compounds have been determined in rats and mice (Domingo et al. 1988a). In general, mice appear to be more sensitive than rats to the lethal effects of zinc. In rats, zinc acetate was the most lethal compound tested; zinc nitrate, zinc chloride, and zinc sulfate (in order of decreasing toxicity) were less lethal. In mice, the most lethal compound was zinc acetate followed by zinc nitrate, zinc sulfate, and zinc chloride. Ingestion of 390 mg zinc/kg/day as zinc oxide in the diet for 3-13 days was lethal to ferrets (Straube et al. 1980). An equivalent dose in humans would be approximately 27 g zinc/day (which would probably be intolerable to humans

because of gastric discomfort). Death was reported in mice that consumed 1,110 mg zinc/kg/day as zinc sulfate in their diet for 13 weeks (Maita et al. 1981). Mortality was also observed in 20% of rats ingesting 191 mg zinc/kg/day as zinc acetate in drinking water for 3 months (Llobet et al. 1988a).

The LD_{50} values and all LOAEL values from each reliable study for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

Ingestion of zinc or zinc-containing compounds has resulted in a variety of systemic effects in the gastrointestinal and hematological systems and alterations in the blood lipid profile in humans and animals. In addition, lesions have been observed in the liver, pancreas, and kidneys of animals. No studies were located regarding respiratory effects in humans or animals after oral exposure to zinc.

Observed systemic effects after oral exposure are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2. The effects discussed in case reports are not included in Table 2-2 or Figure 2-2 because of the small sample size and lack of control data.

Cardiovascular Effects. A number of studies in humans and animals have examined the effects of zinc on serum cholesterol and triglycerides. These data are discussed below under Other Systemic Effects. However, no studies regarding the direct relationship between excessive zinc intake and cardiac mortality were located. No effects on electrocardiographic results were found in a group of elderly subjects (>65 years of age) taking zinc supplements of up to 2 mg zinc/kg/day (Hale et al. 1988) or 0.71 mg zinc/kg/day (Czerwinski et al. 1974). There was also no effect on the frequency of cardiovascular disease (heart attack, heart failure, hypertension, or angina) in elderly subjects (>67 years of age) taking up to 2 mg zinc/kg/day (Hale et al. 198s).

In one study, patients having inoperable severe occlusive vascular disease were administered 3.8 mg zinc/kg/day as zinc sulfate for at least 1 year (Henzel et al. 1971). Eighteen of the

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
ACUTE EXPOSURE									
Death									
1	Rat	(G)	Once				237 (LD50)	Domingo et al. 1988a	acetate
2	Rat	(G)	Once				623 (LD50)	Domingo et al. 1988a	sulfate
3	Rat	(G)	Once				528 (LD50)	Domingo et al. 1988a	chloride
4	Rat	(G)	Once				293 (LD50)	Domingo et al. 1988a	nitrate
5	Mouse	(G)	Once				337 (LD50)	Domingo et al. 1988a	sulfate
6	Mouse	(G)	Once				86 (LD50)	Domingo et al. 1988a	acetate
7	Mouse	(G)	Once				605 (LD50)	Domingo et al. 1988a	chloride
8	Mouse	(G)	Once				204 (LD50)	Domingo et al. 1988a	nitrate
9	Ferret	(F)	<2 wk				390 (3/3 died)	Straube et al. 1980	oxide
Systemic									
10	Human	(W)	Once	Other Other (serum glucose and insulin)	0.5	0.5 (decreased serum cortisol levels)		Brandao-Neto et al. 1990a	sulfate
11	Human	(W)	Once	Gastro		6.7 (gastrointestinal distress; diarrhea)		Callender and Gentzkow 1937	oxide

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
12	Human	(F)	2 d	Gastro Other	86	86 (increased serum amylase, lipase)		Murphy 1970	elemental
Neurological									
13	Rat	(G)	10 d 1x/d			487 (minor neuronal degeneration; decreased acid phosphatase and acetylcholinesterase; increased thiamine pyrophosphatase)		Kozik et al. 1980	oxide
INTERMEDIATE EXPOSURE									
Death									
14	Rat	(W)	3 mo ad lib				191 (2/10 died)	Llobet et al. 1988a	acetate
15	Mouse	(F)	13 wk ad lib				1110 (5/24 died)	Maita et al. 1981	sulfate
Systemic									
16	Human	(C)	12 wk 1x/d	Other		0.71 (decreased serum HDL-cholesterol)		Black et al. 1988	gluconate
17	Human	(C)	5 wk 2x/d	Other		2.3 (decreased serum HDL-cholesterol)		Hooper et al. 1980	sulfate
18	Human	(C)	10 wk 7d/wk 2x/d	Hemato		0.83[b] (decreased superoxide dismutase activity, hematocrit, and serum ferritin)		Yadrick et al. 1989	gluconate

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
19	Human	(F)	6 wk 3x/d	Gastro		2.0 (abdominal cramps; vomiting; nausea)		Samman and Roberts 1987	sulfate
20	Human	(C)	6 wk 7d/wk 3x/d	Other (HDL-cholesterol, LDL-cholesterol)	2.4			Samman and Roberts 1988	sulfate
21	Human	(C)	6 wk 7d/wk 2x/d	Hemato		0.71 (decreased superoxide dismutase activity)		Fischer et al. 1984	gluconate
22	Human	(C)	24 wk 7d/wk 3x/d	Cardio	0.71			Czerwinski et al. 1974	sulfate
23	Human	(C)	3 mo 7d/wk 1x/d	Other (HDL-cholesterol)	1.5			Bogden et al. 1988	acetate
24	Human	(C)	6 wk 2x/d	Other		4.3 (increased serum LDL-cholesterol; decreased serum HDL-cholesterol)		Chandra 1984	sulfate
25	Rat	(F)	13 wk ad lib	Gastro	565			Maita et al. 1981	sulfate
				Hemato	53	565 (decreased hematocrit and WBC)			
				Musc/skel	565				
				Renal	565				
				Other	53		565 (acinar cell necrosis and metaplasia in pancreas)		
26	Rat	(F)	6 wk 7d/wk ad lib	Hemato		6 (ceruloplasmin reduced by 28%)		L'Abbe and Fischer 1984a	sulfate

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
27	Rat	(W)	4 wk 7d/wk ad lib	Hemato		12 (decreased Hb and erythrocytes)		Zaporowska and Wasilewski 1992	chloride
28	Rat	(F)	5 wk ad lib	Hemato		500 (decreased Hb, hematocrit, MCH, MCHC; slightly increased WBC)		Smith and Larson 1946	carbonate
29	Rat	(W)	3 mo ad lib	Hemato	191			Llobet et al. 1988a	acetate
				Hepatic	191				
				Renal	95		191 (increased plasma creatinine and urea levels; desquamation of epithelial cells of proximal tubules)		
				Other (body weight)	191				
30	Rat	(F)	6 wk ad lib	Hemato		350 (decreased Hb)		Smith and Larson 1946	carbonate
31	Rabbit	(F)	22 wk daily	Hemato		174 (slight decrease in Hb levels)		Bentley and Grubb 1991	carbonate
				Other (body weight)	174				
32	Mouse	(F)	13 wk ad lib	Gastro	104		1110 (ulceration of forestomach)	Maita et al. 1981	sulfate
				Hemato	104	1110 (decreased WBC; anemia)			
				Renal	104	1110 (unspecified regressive lesions)			
				Other	104		1110 (acinar cell necrosis and metaplasia in pancreas)		
33	Mouse	(F)	9 mo ad lib	Hemato			68 (severe anemia)	Walters and Roe 1965	oleate

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
34	Dog	(W)	9 mo ad lib	Musc/skel	4			Anderson and Danylchuk 1979	oxide
35	Ferret	(F)	7-97 d ad lib	Gastro	195		390 (intestinal hemorrhages)	Straube et al. 1980	oxide
				Hemato	65	195 (anemia)			
				Renal	65	195 (nephrosis)			
				Other	195	390 (pancreatitis)			
36	Mink	(F)	144 d ppd70-214	Hemato	323.6			Aulerich et al. 1991	sulfate
				Hepatic	323.6				
				Renal	323.6				
				Other (body weight)	323.6				
37	Cow	(F)	5 wk 2x/d ppd3-40	Hemato		64 (decreased hematocrit levels)		Jenkins and Hidiroglou 1991	oxide
				Other	64		91 (body weight gain decreased 46%)		
Immunological									
38	Human	(C)	6 wk 2x/d			4.3 (impaired lymphocyte and polymorphonuclear leukocyte function)		Chandra 1984	sulfate
39	Human	(C)	3 mo 7d/wk 1x/d		1.5			Bogden et al. 1988	acetate
40	Human	(C)	1 mo 2x/d		2.5			Duchateau et al. 1981	sulfate
41	Mouse	(F)	4 wk 7d/wk ad lib		76.9			Schiffer et al. 1991	sulfate
42	Mouse	(F)	8 wk 7d/wk ad lib		6.5			Fernandes et al. 1979	NS

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
Developmental									
43	Human	(C)	Gwk 20 through parturition		0.3			Mahomed et al. 1989	sulfate
44	Human	(C)	last 15-25 wk of pregnancy 1x/d		0.3			Simmer et al. 1991	citrate
45	Human	(C)	11 wk 1x/d		0.06			Kynast and Saling 1986	aspartate
46	Rat	(F)	20 d Gd0-20 ad lib		25			Uriu-Hare et al. 1989	carbonate
47	Rat	(F)	15 d Gd1-15 ad lib				200 (29% fetal resorption; decreased fetal weight)	Schlicker and Cox 1968	oxide
48	Rat	(F)	36 d Gd1-15 ad lib		100			Schlicker and Cox 1968	oxide
49	Rat	(F)	150 d ad lib		50		250 (increased stillbirths)	Sutton and Nelson 1937	carbonate
50	Rat	(F)	7 wk Gd0-17 ad lib		250			Kinnamon 1963	carbonate
51	Rat	(F)	36 d Gd1-21 ad lib				200 (100% fetal resorption)	Schlicker and Cox 1968	oxide
52	Mouse	(F)	2 gen			260 (alopecia; decreased hematocrit)		Mulhern et al. 1986	carbonate

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
53	Mink	(F)	approx 25 wk ad lib		20.8			Bleavins et al. 1983	sulfate
Reproductive									
54	Human	(C)	Gwk 20 through parturi-tion		0.3			Mahomed et al. 1989	sulfate
55	Rat	(F)	150 d ad lib		50		250 (no reproduction in females)	Sutton and Nelson 1937	carbonate
56	Rat	(F)	8 wk 7d/wk ad lib			25 (altered sperm chromatin structure)		Evenson et al. 1993	chloride
57	Rat	(F)	18 d Gd0-18 ad lib				200 (increased pre-implantation loss)	Pal and Pal 1987	sulfate
58	Mouse	(F)	13 wk ad lib		1110			Maita et al. 1981	sulfate
59	Mink	(F)	approx 25 wk ad lib		20.8			Bleavins et al. 1983	sulfate
CHRONIC EXPOSURE									
Systemic									
60	Human	(C)	8 yr 7d/wk 1x/d	Cardio Hemato Other (cholesterol)	2.0 2.0	 2.0 (decreased RBC) 		Hale et al. 1988	NS

TABLE 2-2. Levels of Significant Exposure to Zinc - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg Zn/kg/day)	LOAEL (effect) Less serious (mg Zn/kg/day)	LOAEL (effect) Serious (mg Zn/kg/day)	Reference	Form
61	Mouse	(W)	5-14 mo ad lib	Other		70 (hypertrophy and vacuolation of pancreas islet cells; hypertrophy and vacuolization of fasciculata cells in adrenal cortex)		Aughey et al. 1977	sulfate

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive intermediate oral minimal risk level (MRL) of 0.3 mg/kg/day. The sum of the reported supplemental dose (0.83 mg/kg/day) and the estimate from the FDA Total Diet Study for 1982-1986 (0.16 mg/kg/day) resulted in a total dose of 1 mg/kg/day. The total dose was then divided by an uncertainty factor of 3 (based on minimal LOAEL from a study of the most sensitive humans and the consideration that zinc is an essential dietary nutrient). The intermediate oral MRL was adopted as the chronic oral MRL.

acetate = zinc acetate; ad lib = ad libitum; approx = approximately; aspartate = zinc aspartate; (C) = capsule; carbonate = zinc carbonate; Cardio = cardiovascular; chloride = zinc chloride; citrate = zinc citrate; d = day(s); elemental = elemental zinc; (F) = feed; (G) = gavage, not specified; Gastro = gastrointestinal; Gd = gestation day; gen = generation; gluconate = zinc gluconate; Gwk = gestation week; Hb = hemoglobin; HDL = high density lipoprotein; Hemato = hematological; LD50 = lethal dose, 50% kill; LDL = low density lipoprotein; LOAEL = lowest-observed-adverse-effect level; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; mo = month(s); Musc/skel = musculoskeletal; nitrate = zinc nitrate; NOAEL = no-observed-adverse-effect level; NS = not specified; oleate = zinc oleate; oxide = zinc oxide; ppd = post partum day; RBC = red blood cell; sulfate = zinc sulfate; (W) = drinking water; WBC = white blood cell; wk = week(s); x = time(s); yr = year(s); Zn = zinc

FIGURE 2-2. Levels of Significant Exposure to Zinc - Oral

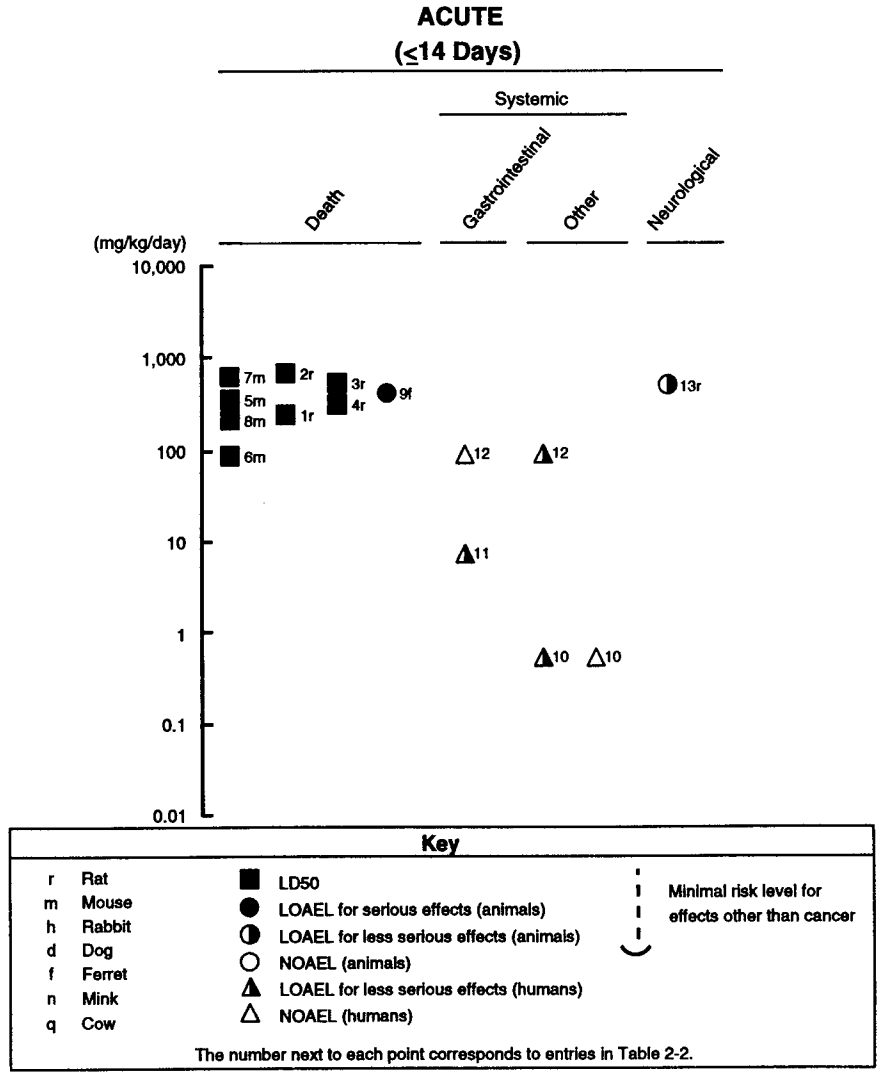


FIGURE 2-2. Levels of Significant Exposure to Zinc - Oral *(continued)*

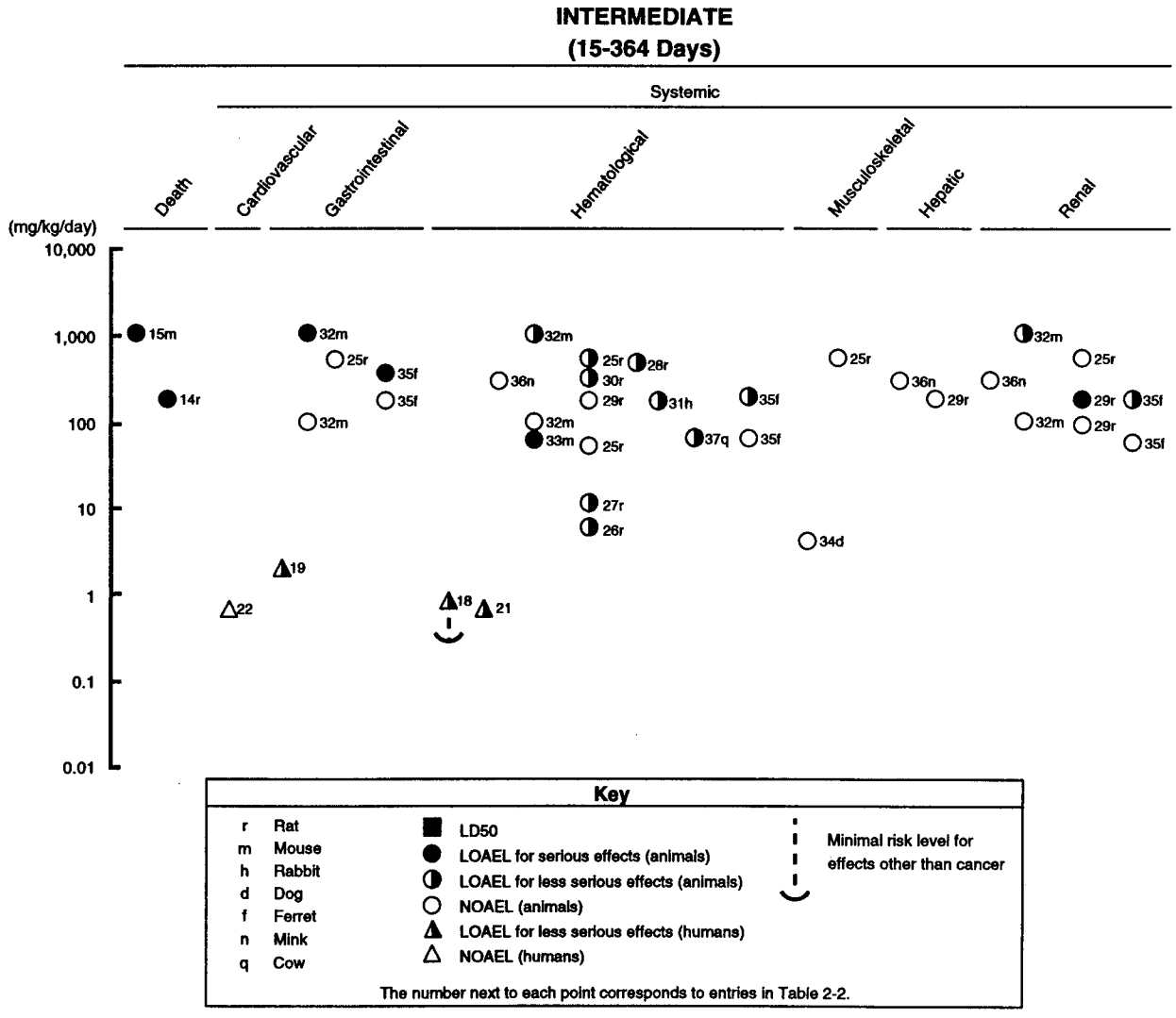


FIGURE 2-2. Levels of Significant Exposure to Zinc - Oral *(continued)*

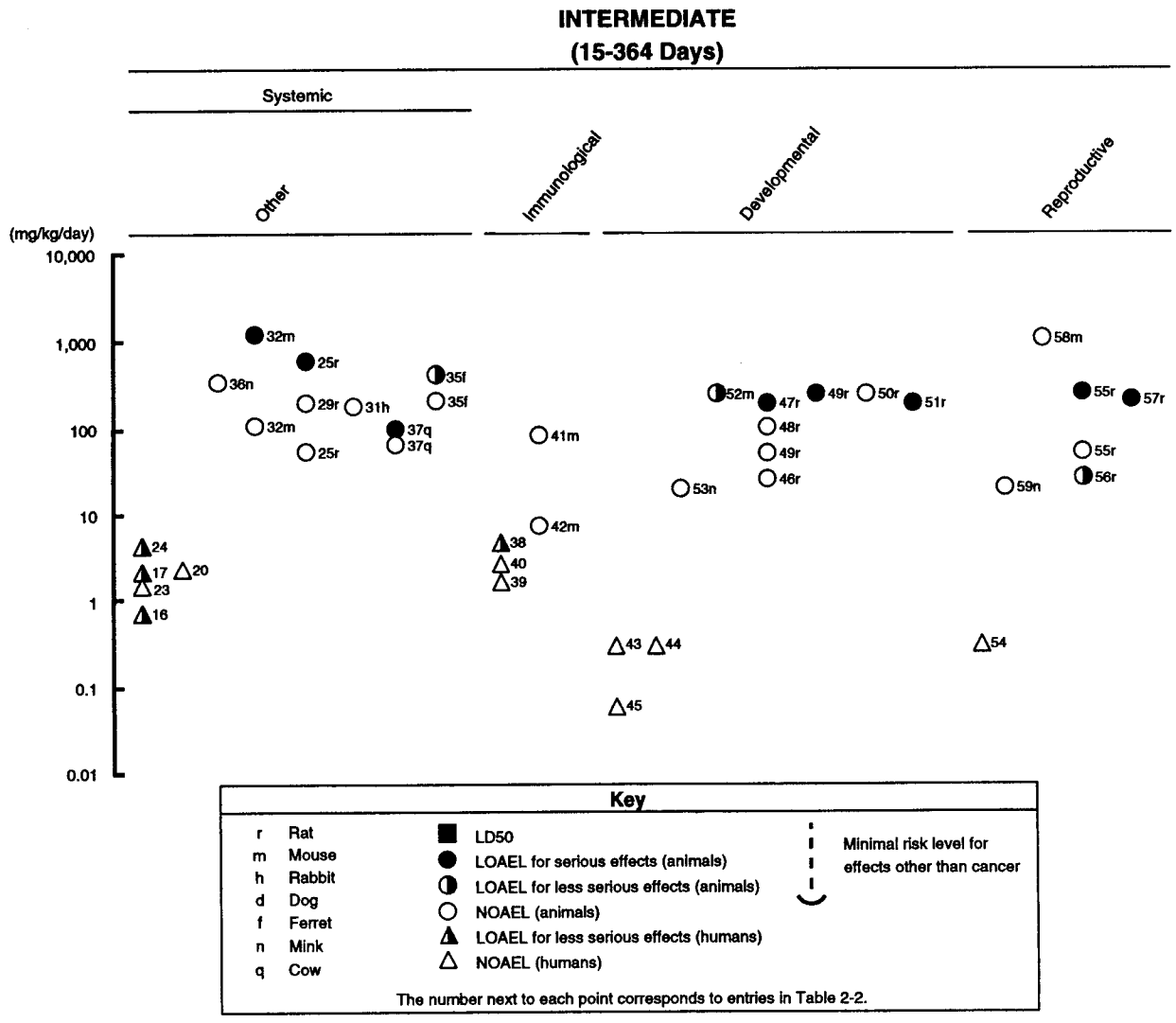
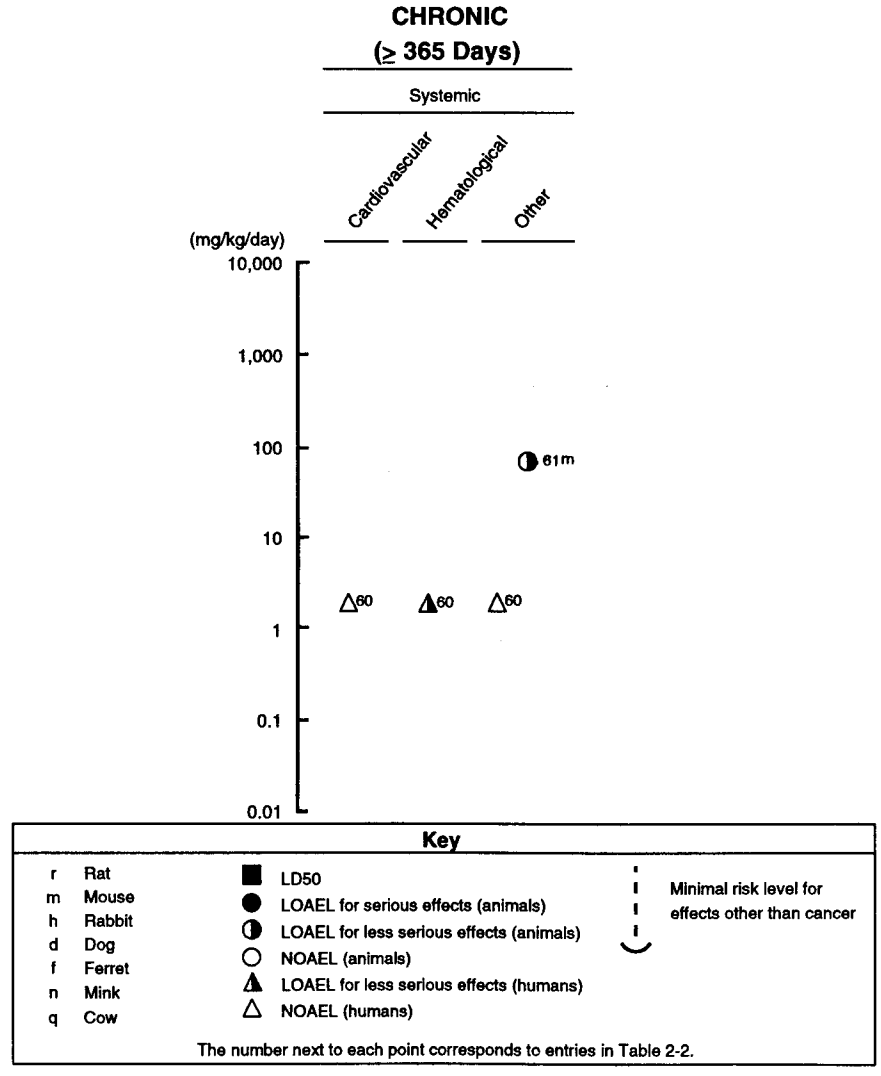


FIGURE 2-2. Levels of Significant Exposure to Zinc - Oral *(continued)*



24 patients experienced improvement in lower extremity blood flow and unchanged or decreased arterial pressure. Zinc's role in these improvements was not completely understood by the study authors. They hypothesized that when optimal zinc levels are provided to the ischemic limb, the activity of certain zinc enzymes promotes the reversal of tissue-dependent hypoxia and/or lactic acidemia in the muscles. It is also not known if this high dose of zinc was associated with any toxic effects.

No studies were located regarding cardiovascular effects in animals after oral exposure to zinc.

Gastrointestinal Effects. Several studies have suggested that zinc ingestion may cause symptoms of gastrointestinal distress or alterations in gastrointestinal tissues. For example, one individual who ingested about 3 ounces of a zinc chloride solution described acute symptoms that occurred almost immediately following contact with the compound, including burning and pain in the mouth and throat and vomiting (Chobanian 1981). Later, the patient exhibited pharyngitis, esophagitis, hypocalcemia, and elevated levels of amylase; the latter two alterations are suggestive of acute pancreatitis. The patient received intravenous hydration and calcium supplementation and recovered within 5 days. The material ingested was described as a "zinc chloride solution," and its concentration was not reported. Therefore, a dose level could not be established in this case.

Several cases of gastrointestinal disturbances have been reported after ingestion of large amounts of zinc as zinc sulfate (Anonymous 1983; Brown et al. 1964; Moore 1978; Samman and Roberts 1987). Vomiting, abdominal cramps, and diarrhea, in several cases with blood, have been observed after ingestion of zinc sulfate. In one report, an English school girl ingested 440 mg zinc sulfate/day (2.6 mg zinc/kg/day) in capsules as a medically prescribed treatment for acne (Moore 1978). After taking each capsule, she experienced epigastric discomfort. A week later, she was admitted to the hospital after a fainting spell. She was diagnosed as anemic and subsequently passed melanic stools, indicative of gastrointestinal bleeding. Gastrointestinal upset (abdominal cramps, vomiting, nausea) occurred in 26 of 47 healthy volunteers following ingestion of zinc sulfate tablets (150 mg as zinc ion in three divided doses per day, 2 mg zinc/kg/day) for 6 weeks (Samman and Roberts 1987). Ingestion of zinc oxide has also been associated with gastrointestinal distress (Anonymous 1983; Callender and Gentzkow 1937). In one case, 80% of the personnel of two army companies became ill with gastrointestinal distress and diarrhea after

consuming limeade prepared in galvanized trash cans (Callender and Gentzkow 1937). The average dose was estimated to be 6.7-7.1 mg/kg. A second example was presented in a case involving school children in New Mexico who experienced nausea and vomiting after accidental excessive zinc intake (Anonymous 1983). These children had consumed punch containing high levels of zinc dissolved from galvanized hinges attached to tanks in which the punch was stored. A 16-year-old boy who ingested 12 g elemental zinc over a 2-day period (86 mg zinc/kg/day) experienced light-headedness, lethargy, staggering gait, and difficulty writing legibly, but no apparent gastrointestinal disturbances (Murphy 1970).

Gastrointestinal effects have also been observed in animals. Intestinal hemorrhages were observed in ferrets that ingested 390 mg zinc/kg/day as zinc oxide for 2 weeks (Straube et al. 1980). These ferrets exhibited a 75% reduction in food intake. No intestinal hemorrhaging was observed in ferrets fed 195 mg/kg/day for up to 21 days. Oral zinc sulfate exposures of intermediate duration in other experimental animals have also resulted in gastrointestinal effects. Mice fed a diet providing 1,110 mg zinc/kg/day developed ulcers in the forestomach, but gastrointestinal effects were not observed in rats fed 565 mg zinc/kg/day (Maita et al. 1981).

Hematological Effects. In a case report, acute exposure to 2.6 mg zinc/kg/day as zinc sulfate for 1 week resulted in anemia (Moore 1978). The authors of the report noted that the anemia may have been secondary to the gastrointestinal hemorrhages.

Treatment-related changes in hematological parameters have been observed in humans and animals after intermediate or chronic exposure to zinc or zinc-containing compounds. Long-term administration (l-8 years) of zinc supplements has caused anemia in humans (Broun et al. 1990; Gyorffy and Chan 1992; Hale et al. 1988; Hoffman et al. 1988; Patterson et al. 1985; Porter et al. 1977; Prasad et al. 1978; Ramdurai et al. 1993; Stroud 1991; Summerfield et al. 1992). Exposure to 2 mg zinc/kg/day as zinc sulfate for 10 months resulted in anemia (Hoffman et al. 1988). A significant reduction in erythrocyte superoxide dismutase activity (47% decrease), hematocrit, and serum ferritin, compared to pretreatment levels, occurred in female subjects who received supplements (as capsules) of 50 mg zinc/day as zinc gluconate for 10 weeks (Yadrick et al. 1989). A 15% decrease in erythrocyte superoxide dismutase activity was reported in male volunteers receiving 50 mg zinc/day as zinc gluconate for 6 weeks (Fisher et al. 1984).

In animals, following oral administration of zinc compounds, decreased hemoglobin, hematocrit, erythrocyte, and/or leukocyte levels were observed in rats (Maita et al. 1981; Smith and Larson 1946), mice (Maita et al. 1981; Walters and Roe 1965) rabbits (Bentley and Grubb 1991) dogs (Drinker et al. 1927d; Meurs et al. 1991; Robinson et al. 1991), ferrets (Straube et al. 1980), and preruminant calves (Jenkins and Hidiroglou 1991). In rats, the lowest LOAEL for decreased hemoglobin (85% of control value) is 12 mg zinc/kg/day as zinc chloride in a 4-week drinking water study with 2-month-old rats (Zaporowska and Wasilewski 1992). The highest NOAEL in rats is 191 mg zinc/kg/day as zinc acetate in a 3-month drinking water study (age of rats not specified) (Llobet et al. 1988a). The reason that the lowest LOAEL is less than the highest NOAEL in rats is unclear, but it may be because of the use of different zinc compounds or different rat strains or age. The second lowest rat LOAEL is 350 mg zinc/kg/day as zinc carbonate (Smith and Larson 1946). For mice, NOAEL and LOAEL values of 104 and 1,110 mg zinc/kg/day as zinc sulfate, respectively, were identified by Maita et al. (1981) in a 13-week feeding study. A LOAEL of 68 mg zinc/kg/day as zinc oleate was observed in a 9-month mouse feeding study (Walters and Roe 1965). It is not known if the difference in the LOAELs identified in the Maita et al. (1981) and Walters and Roe (1965) studies is due to the use of different zinc compounds, different basic diet formulations, different mouse strains, or different exposure durations. Slight decreases in hemoglobin levels were observed in rabbits fed 174 mg zinc/kg/day as zinc carbonate (Bentley and Grubb 1991). Zinc oxide consumption caused anemia in dogs (76.5 mg zinc/kg/day) (Drinker et al. 1927d), ferrets (195 mg zinc/kg/day) (Straube et al. 1980) and preruminant calves (64 mg zinc/kg/day) (Jenkins and Hidiroglou 1991). Hematological alterations were not observed in cats exposed to up to 83.2 mg zinc/kg/day as zinc oxide (Drinker et al. 1927d) or in adult mink exposed to zinc at up to 297.4 mg zinc/kg/day as zinc oxide (Aulerich et al. 1991; Bleavins et al. 1983). However, decreases in hematocrit and lymphocytes were observed in the offspring of mink females that ingested a time-weighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to conception and throughout gestation and lactation (Bleavins et al. 1983) indicating that very young mink may be more sensitive to the hematologic effects of zinc than adults. An increased number of weanling rats had low levels of ceruloplasmin, a copper serum protein, after administration of zinc sulfate for 6 weeks (Abbe and Fischer 1984a).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to zinc.

Rib biopsies revealed no treatment-related effects in dogs given 4 mg zinc/kg/day as zinc oxide in the diet for 9 months (Anderson and Danylchuk 1979).

Hepatic Effects. Ingestion of 3.5 mg/kg/day zinc sulfate for 18 weeks by 13 patients being treated for chronic venous leg ulcers was reported to have no effect on the results of liver function tests (Hallbook and Lanner 1972). However, the type of liver function tests was not specified and results were not presented to support this conclusion.

No histopathology or changes in serum enzyme levels (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, or alkaline phosphatase) were observed in rats receiving 191 mg zinc/kg/day as zinc acetate (Llobet et al. 1988a). Similarly, no histopathology was observed in rats administered 98.3 mg zinc/kg/day as zinc oxide, but an insufficient number of animals were tested (Drinker et al. 1927c). Sheep fed time-weighted-average doses of 19 mg zinc/kg/day as zinc oxide for 49-72 days developed hepatic effects, including necrotic hepatocytes and large quantities of hemosiderin in Kupffer cells (Allen et al. 1983). Because sheep are ruminants, it is not known if they are a good model for predicting human toxicity. No histological damage was observed in adult or young mink fed 164.8 or 297.4 mg zinc/kg/day, respectively, as zinc sulfate for 144 days (Aulerich et al. 1991).

Decreased hexobarbital sleeping times were reported by Kadiiska et al. (1985) in rats receiving 40 mg zinc/kg/day as zinc sulfate. This physiological response suggested an induction of microsomal enzymes.

Renal Effects. Thirteen patients treated with zinc sulfate at 3.5 mg zinc/kg/day for 18 weeks for chronic venous leg ulcers had normal urinalyses (Hallbook and Lanner 1972). However, neither the specific parameters measured for the urinalysis nor the results were presented to support this conclusion. Furthermore, urinalysis may not be a sensitive indicator of renal function.

A number of intermediate-duration studies have demonstrated renal effects in animals exposed to zinc oxide, zinc sulfate, and zinc acetate. Zinc sulfate caused an increase in the absolute and relative kidney weights and regressive kidney lesions (not specified) in female mice that consumed 1,110 mg zinc/kg/day in the diet for 13 weeks, but no effects occurred in rats that consumed 565 mg zinc/kg/day under similar conditions (Maita et al. 1981). Severe diffuse

nephrosis was observed in ferrets exposed to 195 mg zinc/kg/day as zinc oxide in the diet (Straube et al. 1980). In rats exposed to 191 mg zinc/kg/day as zinc acetate for 3 months, epithelial cell damage in the glomerulus and proximal convoluted tubules and increased plasma creatinine and urea levels were observed (Llobet et al. 1988a). The NOAEL for the effects on creatinine and urea was 95 mg zinc/kg/day. It is unclear whether the microscopic changes were observed at lower doses. No histopathological changes in the kidneys were observed in three rats that drank water containing 98.3 mg zinc/kg/day as zinc oxide for 35-36 weeks (Drinker et al. 1927c); however, interpretation of the results of this study is severely limited by the small number of rats used. Renal tubular dilation, with proteinaceous casts and hemosiderin deposits, was observed in the kidneys of sheep that ingested 18 mg zinc/kg/day as zinc oxide for 49-72 days (Allen et al. 1983). It is not known if sheep are a good model for human toxicity because they are ruminants. No renal effects were observed in either adult mink consuming 326.7 mg zinc/kg/day as zinc sulfate or in young mink consuming 323.6 mg zinc/kg/day as zinc sulfate for 144 days (Aulerich et al. 1991).

Dermal/Ocular Effects. No studies were located regarding dermal/ocular effects in humans after oral exposure to zinc.

No dermal effects were seen in female minks given a time-weighted dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to mating and then throughout gestation and lactation (Bleavins et al. 1983).

Other Systemic Effects

Pancreas. Increased levels of serum amylase were observed in a man after accidental ingestion of about 3 ounces of a zinc chloride solution (Chobanian 1981). A 16-year-old boy who ingested an average of approximately 86 mg zinc/kg/day as metallic zinc for 2 days (114 mg/kg on the 1st day and 57 mg/kg on the 2nd day) had increased serum amylase and lipase (Murphy 1970).

In humans receiving a single low dose of zinc sulfate (0.5 mg zinc/kg/day), no changes in blood glucose or insulin levels were observed, and there were no differences in response to a glucose load (Brandao-Neto et al. 1990b).

Pancreatic abnormalities (islet cellular alterations, acinar cell necrosis, metaplasia, fibrosis, pancreatitis) resulting from zinc ingestion have been observed in rats (Maita et al. 1981) mice (Aughey et al. 1977; Maita et al. 1981), cats (Drinker et al. 1927d), ferrets (Straube et al. 1980) sheep (Allen et al. 1983), and birds (Kazacos and Van Vleet 1989; Lu et al. 1990). In dogs (Drinker et al. 1927d) and minks (Aulerich et al. I991), histological changes in the pancreas have not been observed at doses comparable to or higher than the dose levels that caused abnormalities in rats, mice, cats, ferrets, and sheep. Degeneration of the acinar cells of the pancreas was observed in sheep by Allen et al. (1983) and in rats and mice by Maita et al. (1981). Since the pancreatic acinar cells secrete digestive juices into the small intestine, the increase in serum amylase and lipase observed in the human case reports (Chobanian 1981; Murphy 1970) would correspond to damage in this region of the pancreas.

In 2-month-old C3H mice exposed to 70 mg zinc/kg/day as zinc sulfate, hypertrophy and vacuolation of the β-cells of the pancreatic islets were observed beginning after 3 months of exposure and become more severe by 12 months (Aughey et al. 1977). The pancreatic islets secrete the hormones glucagon and insulin. No change in plasma levels of insulin and glucose was observed in this study after 6 months of exposure. No effect on islet cells was reported in rats exposed up to 565 mg/kg/day or mice exposed to 1110 mg/kg/day as zinc sulfate in a 13-week study by Maita et al. (1981) and Allen et al. (1983) reported that islet cells in sheep were generally unaffected, although occasional vacuolization occurred. Degeneration of acinar cells, but no effects on the islet cells, were found in ducklings (Kazacos and Van Vleet 1989); however, the relevance of this to humans is unclear. The data are too limited and contradictory to determine whether pancreatic islet cells are a primary target cell of zinc toxicity.

Adrenal Gland. Decreased levels of serum cortisol (a hormone secreted by the adrenal cortex) were observed in humans after a single dose of 0.5 mg zinc/kg/day as zinc sulfate (Brandao-Neto et al. 1990b). No effects on the adrenal gland itself have been reported in humans.
In mice receiving 70 mg zinc/kg/day as zinc sulfate in the drinking water, hypertrophy and increased lipid content of the zona fasciculata cells of the adrenal cortex were observed as early as 3 months after the start of zinc supplementation (Aughey et al. 1977).

Pituitary. No effects on pituitary function have been reported in humans following oral exposure to zinc. However, mice receiving 70 mg zinc/kg/day as zinc sulfate in the drinking water for 5-14 months had hypertrophy and increased granularity suggesting increased activity of the pituitary (Aughey et al. 1977). It is unclear whether the increased activity was a direct effect of the zinc or a reaction to decreased secretion from the adrenal cortex.

Serum Lipid Levels. Several reports described changes in the serum lipid profile of humans exposed to zinc sulfate or gluconate for 3-12 months; however, the results are mixed. Ingestion of 2.3-4.3 mg zinc/kg/day for 5-6 weeks (Chandra 1984; Hooper et al. 1980) or 0.71 mg zinc/kg/day for 12 weeks (Black et al. 1988) reduced levels of high-density lipoprotein (HDL) cholesterol. In the study by Chandra (1984) a slight increase in low-density lipoprotein (LDL) cholesterol was observed in subjects who served as their own controls; measurements were taken prior to zinc supplementation and after a lo-week postexposure period. Serum cholesterol, triglyceride, and LDL cholesterol levels were not affected by zinc supplementation in the study by Black et al. (1988). However, in another study, zinc supplements depressed HDL cholesterol levels and raised LDL cholesterol levels in elderly subjects (>60 years of age), especially in those who exercised. This study was not well controlled, and the wide variation in doses of the supplemented group prevented the determination of a LOAEL (Goodwin et al. 1985). Young women with a total daily intake of 1.6 mg zinc/kg/day in a 2-month study had a transient decrease in HDL cholesterol (Freeland-Graves et al. 1980). In a double-blind crossover study of young men and women receiving 2.0 (men) or 2.4 (women) mg zinc/kg/day for 6 weeks, total HDL cholesterol was not affected, and LDL cholesterol was significantly decreased in the women (Samman and Roberts 1988). No effect on HDL cholesterol was seen in elderly men and women (60-89-years old) with a total daily intake (dietary zinc plus a zinc acetate supplement) of 1.5 mg/kg/day for 3 months (Bogden et al. 1988) but the subjects also received copper supplements (about 0.03 mg/kg). Another study (Hale et al. 1988) reported no differences in triglycerides and cholesterol levels in subjects (>68-years old) given zinc supplements of up to 2 mg/kg/day for an average of 8 years.

Increases in serum cholesterol levels were observed in two studies where rats were fed either 2.8 or 10 mg zinc/kg/day as zinc acetate for 2-7 months (Katya-Katya et al. 1984; Klevay and Hyg 1973). Other studies have shown no effect on total cholesterol, HDL cholesterol, or serum

triglyceride levels in rats ingesting 3 or 2.5 mg zinc/kg/day of unspecified zinc compounds (Fischer et al. 1980; Woo et al. 1983).

Body Weight. No effects on body weight have been reported in humans following oral exposure to zinc. However, body weight gain was decreased by 46% in preruminant calves that consumed 91 mg zinc/kg/day as zinc oxide for 5 weeks; there was no effect at 64 mg zinc/kg/day (Jenkins and Hidiroglou 1991). The relevance of this effect to humans is unclear. Body weights of rabbits-(Bentley and Grubb 1991), rats (Llobet et al. 1988a), and minks (Aulerich et al. 1991) were unaffected by dosing with 174, 191, and 326.7 mg zinc/kg/day, respectively, for 3-12 months.

2.2.2.3 Immunological Effects

Zinc plays a role in the normal development and maintenance of the immune system, such as in the lymphocyte response to mitogens and as a cofactor for the thymic hormone thymulin (Delafuente 1991; Franker et al. 1986). Oral exposure to zinc at levels much higher than the RDA has impaired immune and inflammatory responses. This was observed in *in vivo* investigations of the immune competence of blood components taken from 11 healthy adult men after ingestion of 4.3 mg zinc/kg/day as zinc sulfate for 6 weeks. The mitogenic response elicited from peripheral blood lymphocytes and the chemotactic and phagocytic responses of polymorphonuclear leukocytes were impaired after zinc ingestion. No effects were seen on total numbers of lymphocytes or relative numbers of T cells, T cell subsets, or B cells (Chandra 1984). The relationship between these observations and decreased levels of immune competence that might lead to increased susceptibility to disease is unknown. Zinc supplements administered to elderly populations at doses up to 1.5 mg zinc/kg/day (Bogden et al. 1988) or 2.5 mg zinc/kg/day (Duchateau et al. 1981) resulted in either no effect or a beneficial effect on immune cell titers or delayed cutaneous hypersensitivity responses to specific antigens.

Decreased lymphocyte activity (incorporation of ^{3}H-thymidine in response to concanavalin A) was reported in mink kits from dams who had ingested a time-weighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to conception and throughout gestation and lactation (Bleavins et al. 1983). The dose to the kits is unknown. In contrast, no effect was observed on antibody titre (immunoglobulin G [IgG] and immunoglobulin M [IgM]) or the mitogenic response of splenic B cells isolated from mice fed 76.9 mg zinc/kg/day as zinc sulfate

for 4 weeks and challenged with B cell antigens either *in vivo* or *in vitro* (Schiffer et al. 1991). The *in vitro* mitogenic response of T cells isolated from these mice was increased.

The highest NOAEL value in animals and the LOAEL value in humans for immunological effects after intermediate-duration oral exposure are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

Zinc appears to be necessary for normal brain function (Sandstead et al. 1983), but excess zinc is toxic. A 16-year-old boy who ingested ≈86 mg zinc/kg/day of metallic zinc over a 2-day period in an attempt to promote wound healing, developed signs and symptoms of lethargy, lightheadedness, staggering, and difficulty in writing clearly (Murphy 1970). Lethargy was also observed in a 2-year-old child who ingested a zinc chloride solution (≈l,000 mg zinc/kg) (Potter 1981). It is not known whether these observations represent direct effects on the nervous system.

Very limited data were located regarding neurological effects in animals. Minor neuron degeneration and proliferation of oligodendroglia occurred in rats dosed with 487 mg zinc/kg/day as zinc oxide for 10 days (Kozik et al. 1980). Rats receiving 472 mg zinc/kg/day for 10 days had increased levels of secretory material in the neurosecretory nuclei of the hypothalamus (Kozik et al. 1981).

2.2.2.5 Reproductive Effects

Pregnant women receiving capsules containing 0.3 mg zinc/kg/day as zinc sulfate during the last two trimesters did not exhibit any reproductive effects (no changes in maternal body weight gain, blood pressure, postpartum hemorrhage, or infection) (Mahomed et al. 1989). No other studies were located regarding reproductive effects in humans after oral exposure to zinc.

No measurable effect on gestational length or litter size was observed when female mink ingested a time-weighted'average dose of 20.8 mg zinc/kg/day as zinc sulfate (Bleavins et al. 1983). No histological alterations in the testes or ovaries were noted in mice fed zinc sulfate (1,110 mg zinc/kg/day) for 13 weeks (Maita et al. 1981). Male and female rats receiving 50 mg zinc/kg/day as zinc carbonate in the diet were reported to reproduce normally for several

generations in a poorly documented study by Sutton and Nelson (1937). Rats fed 250 mg zinc/kg/day for 14-17 weeks mated successfully but had a higher than normal percentage of stillborn pups A subsequent mating of the parental generation fed 2.50 mg zinc/kg/day for 5 months was unsuccessful. No reproduction occurred in rats fed 500 mg zinc/kg/day for 5 months (Sutton and Nelson 1937). The frequency of sperm with an altered chromatin structure was increased in rats fed 25 mg zinc/kg/day as zinc chloride for 8 weeks (Evenson et al. 1993). Preimplantation loss increased in rats fed diets containing 200 mg zinc/kg/day as zinc sulfate on gestational days 0-18 (Pal and Pal 1987). When the rats received 200 mg zinc/kg/day 21 days prior to mating, no effects on implantation or other adverse reproductive effects were observed (Pal and Pal 1987). The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

Zinc is necessary for normal fetal growth and development. Fetal damage may result from zinc deficiency. Only one report in the literature suggested adverse developmental effects in humans due to exposure to excessive levels of zinc (Kumar 1976). Four women were given zinc supplements of 0.6 mg zinc/kg/day as zinc sulfate during the third trimester of pregnancy. Three of the women had premature deliveries, and one delivered a stillborn infant. However, the significance of these results cannot be determined because very few details were given regarding the study protocol, reproductive histories, and the nutritional status of the women. Other human studies have found no developmental effects in the newborns of mothers consuming 0.3 mg zinc/kg/day as zinc sulfate (Mahomed et al. 1989) or zinc citrate (Simmer et al. 1991) or 0.06 mg zinc/kg/day as zinc aspartate (Kynast and Saling 1986) during the last two trimesters.

The developmental toxicity of zinc in experimental animals has been evaluated in a number of investigations. Exposure to high levels of zinc in the diet prior to and/or during gestation has been associated with increased fetal resorptions, reduced fetal weights, altered tissue concentrations of fetal iron and copper, and reduced growth in the offspring.

Administration of zinc in rats at 200 mg zinc/kg/day as zinc oxide in the diet for 21 days prior to mating and then throughout gestation resulted in resorption of all fetuses (Schlicker and Cox

1968). Fetal resorptions ranged from 4% to 29% when 200 mg zinc/kg/day was administered only during gestation (controls had no resorptions). When the dose was reduced to 100 mg zinc/kg/day starting 21 days prior to mating, there were no fetal resorptions, malformations, or growth reduction. In contrast, Kinnamon (1963) reported no resorptions, no difference in the number of offspring per litter, and no change in average wet weight of the fetuses from female rats fed 250 mg zinc/kg/day as zinc carbonate in the diet for 53 days before mating and during gestation. The reason for the differences in the results of these studies is unknown. No effect on fetal viability, size, or malformations was seen in fetuses from female rats fed 25 mg zinc/kg/day as zinc carbonate during gestational days l-18 (Uriu-Hare et al. 1989).

Administration of 200 mg zinc/kg/day to dams throughout gestation resulted in decreased growth and tissue levels of copper and iron in fetal rats (Cox et al. 1969; Schlicker and Cox 1968). In rats, at both 100 and 200 mg/kg/day during gestational days l-18, maternal zinc levels increased. However, zinc tissue levels in the 22-day-old fetuses were not elevated at 100 mg/kg/day to dams, suggesting that the placenta was able to act as a barrier to zinc at the lower dietary level. In contrast, Ketcheson et al, (1969) showed that newborn and 14-day-old rats from mothers that had consumed 100 mg/kg/day throughout gestation had elevated levels of total zinc and decreased levels of iron. It is unclear whether the longer exposure to zinc during gestation or the suckling of newborn rats prior to sacrifice may have accounted for these differences.

Animal studies suggest that exposure to very high levels of dietary zinc is associated with reduced fetal weight, alopecia, decreased hematocrit, and copper deficiency in offspring. For example, second generation mice exposed to zinc carbonate during gestation and lactation (260 mg/kg/day in the maternal diet), and then continued on that diet for 8 weeks, had reduced body weight, alopecia, and signs of copper deficiency (e.g., lowered hematocrit and occasional achromotrichia [loss of hair color]) (Mulhern et al. 1986). Similarly, mink kits from dams that ingested a timeweighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate also had alopecia and achromotrichia (Bleavins et al. 1983). It is likely that the alopecia resulted from zinc-induced copper deficiency,which is known to cause alopecia in monkeys (Obeck 1978). However, no adverse effects were observed in parental mice or mink.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxicity in humans after oral exposure to zinc.

Chromosomal aberrations were detected in the bone marrow cells of mice administered 350 mg zinc/kg as zinc chloride and fed a low-calcium diet (1.1% calcium), but not when the animals were given a similar zinc dose and fed a calcium-replete diet (Deknudt and Gerber 1979). A similar effect was observed in rats exposed to 14.8 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 1988).

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

Very limited data were found regarding relationships between the ingestion of zinc and its compounds by humans and the subsequent development of cancer. One study reported an association between an excess rate of gastric cancer in the people of North Wales (Great Britain) and the high zinc-to-copper ratio (≈30:1) in the soil of household gardens (Stocks and Davies 1964). However, the inference that this excess in gastric cancer is causally associated with soil levels of zinc and copper is not consistent with another study. In a survey of cancer registry data (1954-1978) in Shipham, Somerset (Great Britain), an area that also has a high soil zinc-tocopper ratio (≈17:1), the gastric cancer incidence rate was significantly lower than the regional rate (Philipp et al. 1982). It is probable that other factors, not considered by Stocks and Davies (1964), that are associated with or coincidental to the high soil zinc-to-copper ratio confounded the results.

The carcinogenicity of zinc in experimental animals following oral exposure was evaluated by Walters and Roe (1965). The incidence of tumors was not increased in mice exposed to 951 mg zinc/kg/day as zinc sulfate in drinking water for 1 year compared to controls. However, important details regarding the study protocol were lacking including the age and sex of the mice, the number of mice at the beginning of the study, the purity of the test material, and a complete list of the organs and tissues examined at necropsy. The control mice developed intercurrent disease (ectromelia), which resulted in a number of deaths; supplementary control mice were

added to the study, but they were not concurrent controls. The number of animals in treated and control groups surviving at 1 year (study termination) was small (22-28 mice/group). The exposure period (1 year) was less than the standard bioassay period (18-24 months). There were no data in the study (e.g., survival or body weight data) to indicate that a maximum tolerated dose was achieved. These limitations reduce the sensitivity of the study by Walters and Roe (1965) to detect a carcinogenic response.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to zinc.

2.2.3.2 Systemic Effects

Zinc has been reported to promote the healing of burns and wounds when topically applied as zinc oxide or calamine lotion (Gordon et al. 1981). The mechanism by which.this occurs was not discussed by the authors. Zinc oxide contained in an occlusive zinc tape dressing reduced the inflammatory reactions in the granulation tissue of wounded rats (Wetter et al. 1986). The authors speculated that zinc acted either by a continuous release of zinc ions or by modifying components involved in the tape's adhesive properties.

No studies were located regarding respiratory, cardiovascular, gas train tes tin al, musculoskeletal, hepatic, renal, or other systemic effects in humans or animals after dermal exposure to zinc. The systemic effects observed after dermal exposure are discussed below. The NOAEL values and all LOAEL values from each reliable study for dermal effects in each species and duration category are recorded in Table 2-3.

Hematological Effects. A worker who had been employed making up zinc chloride solutions (concentrations not specified) with his hands was found to have microcytic anemia and decreased numbers of platelets (DuBray 1937).

TABLE 2-3. Levels of Significant Exposure to Zinc - Dermal

Species	Exposure duration/ frequency	System	NOAEL ($mg\ Zn/cm^2$)	LOAEL (effect) Less serious ($mg\ Zn/cm^2$)	LOAEL (effect) Serious ($mg\ Zn/cm^2$)	Reference	Form
ACUTE EXPOSURE							
Systemic							
Human	48 hr	Derm/oc	2.9			Agren 1990	oxide
Rabbit	5 d	Derm/oc	0.4			Lansdown 1991	sulfate
Rabbit	5 d	Derm/oc	16			Lansdown 1991	oxide
Rabbit	5 d	Derm/oc		7.2 (slight skin irritation - open patch test)		Lansdown 1991	acetate
		Derm/oc		7.2 (severe skin irritation - occluded patch test)			
Rabbit	5 d	Derm/oc		0.48 (severe skin irritation)		Lansdown 1991	chloride
Gn pig	5 d	Derm/oc	0.4			Lansdown 1991	sulfate
Gn pig	5 d	Derm/oc		0.48 (moderate skin irritation)		Lansdown 1991	chloride
Gn pig	5 d	Derm/oc	16			Lansdown 1991	oxide
Gn pig	5 d	Derm/oc	7.2			Lansdown 1991	acetate
Mouse	5 d	Derm/oc		0.48 (severe skin irritation)		Lansdown 1991	chloride
Mouse	5 d	Derm/oc		0.4 (slight skin irritancy)		Lansdown 1991	sulfate

TABLE 2-3. Levels of Significant Exposure to Zinc - Dermal (continued)

Species	Exposure duration/ frequency	System	NOAEL ($mg\ Zn/cm^2$)	LOAEL (effect) Less serious ($mg\ Zn/cm^2$)	LOAEL (effect) Serious ($mg\ Zn/cm^2$)	Reference	Form
Mouse	5 d	Derm/oc	16			Lansdown 1991	oxide
Mouse	5 d	Derm/oc		7.2 (moderate skin irritation)		Lansdown 1991	acetate

acetate = zinc acetate; chloride = zinc chloride; d = day(s); Derm/oc = dermal/ocular; Gn pig = guinea pig; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; oxide = zinc oxide; sulfate = zinc sulfate; Zn = zinc

No studies were located regarding hematological effects in animals after dermal exposure to zinc.

Dermal/Ocular Effects. No signs of dermal irritation were observed in humans after a 25% zinc oxide patch (2.9 mg/cm^2) was placed on the skin for 48 hours (Agren 1990). However, 14 out of 17 men who were employed in the bagging or packing of zinc oxide and whose skin was frequently covered with zinc oxide dust reported having experienced zinc oxide pox at least once (Turner 1921). The pox appeared as itchy papular-pustular eruptions in the pubic region, scrotum, inner surface of the thigh, and occasionally on the axilla and inner surface of the arms. The study author suggested that these lesions were due to clogging of glands by dust, perspiration, and bacteria when skin surfaces coated with these substances were rubbed together. In contrast, a case study of 24 workers exposed to dusts of either zinc oxide, zinc sulfide, or metallic zinc revealed only 1 worker with papular pustular lesions on the axilla and inner thighs (Batchelor et al. 1926). The difference in the results was attributed to differences in the personal hygiene of the workers in the two studies.

In a case report, accidental splashing of a soldering paste containing 30% zinc chloride into the eye of a plumber produced an immediate reduction in visual acuity, hyperemia, hemorrhaging, conjunctival swelling, cornea1 opacity, bullous keratopathy, and spotting of the lens (Houle and Grant 1973). Most symptoms disappeared after 6 weeks, but residual lens opacities persisted for over a year after the exposure. Reddened conjunctivae and lacrimation were observed in 34 persons who were exposed to extremely high concentrations of zinc chloride smoke when several smoke generators exploded in a tunnel during World War II (Evans 1945). Two of the exposed persons had cornea1 burns and four had small vesicular burns on the forehead or wrist. Zinc chloride was the major component of the smoke. However, other components such as zinc oxide, hexachloroethane, calcium silicide, the igniter, or the heat of the explosion may have contributed to the injuries that were observed.

The dermal irritancy of several zinc compounds was compared in mice, rabbits, and guinea pigs (Lansdown 1991). Of the six zinc compounds tested, zinc chloride had the greatest irritancy potential, followed by zinc acetate and zinc sulfate; no signs of irritation were observed following exposure to zinc oxide. Although zinc chloride is clearly the most irritating, the relative irritancy of zinc sulfate and zinc acetate was not determined because only one dose was tested and a different dose was used for each compound. The severe skin irritancy observed following

application of zinc chloride was characterized by parakeratosis, hyperkeratosis, inflammatory changes in the epidermis and superficial dermis, and acanthosis of the follicular epithelia (Lansdown 1991).

No studies were located regarding the following health effects in humans or animals after dermal exposure to zinc:

2.2.3.3 Immunological Effects
2.2.3.4 Neurological Effects
2.2.3.5 Reproductive Effects
2.2.3.6 Developmental Effects
2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals after dermal exposure to zinc.

2.3 TOXICOKINETICS

There is limited information on the toxicokinetic properties of zinc following inhalation or dermal exposure. Increased zinc levels in the blood and urine of humans and in the tissue of animals after inhalation and dermal exposure to zinc, respectively, indicate that zinc is absorbed by these routes. The toxicokinetic properties of ingested zinc have been extensively studied. The absorption of zinc from the gastrointestinal tract is homeostatically regulated; under normal physiological conditions, 20-30% of ingested zinc is absorbed. Zinc uptake from the intestinal lumen involves passive diffusion and a carrier-mediated process. A number of factors influence the absorption of zinc; these include inhibitors, such as calcium, phosphorus, and dietary fiber and phytates (components of dietary fiber that may coprecipitate with zinc in the intestines), and enhancers, such as amino acids? picolinic acid, and prostaglandin E_2,. Once absorbed, zinc is widely distributed throughout the body. Zinc content is highest in muscle, bone, gastrointestinal

tract, kidney, brain, skin, lung, heart, and pancreas. In plasma, two-thirds of the zinc is bound to albumin which represents the metabolically active pool of zinc. This pool of plasma zinc is frequently referred to as loosely bound zinc because albumin has the ability to give up bound zinc to tissues. Zinc is excreted in both urine and feces.

Metal fume fever, a critical end point, was observed in workers who inhaled high levels of zinc oxide fumes or dust. The mechanism of metal fume fever has been reported to be an immune response to zinc oxide in the respiratory tract. The anemia observed in humans and animals after oral exposure to high levels of zinc could result from a zinc-induced copper deficiency. Excess levels of dietary zinc inhibit the transport of copper to the blood from either the intestinal lumen or the intestinal mucosal cell.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Quantitative studies regarding absorption of zinc and zinc compounds after inhalation exposure in humans are limited. The absorption of inhaled zinc depends on the particle size and solubility. Elevated levels of zinc have been found in the blood and urine of workers exposed to zinc oxide fumes (Hamdi 1969).

The rates or percentages of absorption of inhaled zinc in animals are not available; however, studies provide data on zinc retention in the lungs. Zinc retention values were 19.8%, 11.5%, and 4.7% in the lungs of guinea pigs, rats, and rabbits, respectively, after inhalation exposure (noseonly) to 3.5-9.1 mg zinc/m^3 as zinc oxide aerosol for 2-3 hours (Gordon et al. 1992). The aerosol had a mass median diameter of 0.17 l μm. The retention of zinc in lungs was dose related in male Wistar rats administered a single intratracheal instillation of 0-07-3.7 mg zinc/m^3 as zinc oxide (Hirano et al. 1989). A half-life of 14 hours was calculated for this experiment.

The absorption of zinc oxide fumes lead to increased levels of zinc measured in the liver, kidney, and pancreas of cats exposed to zinc oxide fumes for durations ranging from 15 minutes to 3.25 hours (Drinker and Drinker 1928). The usefulness of the study is limited because reporting was inadequate and particle size of the zinc oxide aerosol was not determined. Some inhaled

particles of zinc oxide are subject to ciliary clearance and swallowing. Thus, a portion of the inhaled zinc may ultimately be absorbed from the gastrointestinal tract.

2.3.1.2 Oral Exposure

Several studies have measured oral absorption rates of zinc in humans. Absorption ranged from 8% to 81% following short-term exposures to zinc supplements in the diet; differences in absorption are probably due to the type of diet (amount of zinc ingested, amount and kind of food eaten) (Aamodt et al. 1983; Hunt et al. 1991; Istfan et al. 1983; Reinhold et al. 1991; Sandstrom and Abrahamson 1989; Sandstrom and Cederblad 1980; Sandstrom and Sandberg 1992). For example, dietary protein facilitates zinc absorption; fractional zinc absorption ranged from 8% for low-protein rolls to 26% for high-protein rolls 3 days after individuals ingested 0.05 mg zinc/kg (Hunt et al. 1991).

Absorption of labeled zinc was 40.0-48.4% in male Wistar rats fed a diet containing 0.81 mg zinc/kg as zinc chloride or zinc carbonate (Galvez-Morros et al. 1992). Fractional absorption in immature organisms generally exceeds that in adults. In growing rats, on the basis of indirect calculation from isotope experiments, Weigand and Kirchgessner (1992) suggested surprisingly high absorption values of as much as 94.7%. It is likely that all these results were influenced by isotope exchange and do not provide estimates of net absorption.

The body's natural homeostatic mechanisms control zinc absorption from the gastrointestinal tract (Davies 1980). Persons with adequate nutritional levels of zinc absorb approximately 20-30% of all ingested zinc. Those who are zinc-deficient absorb greater proportions of administered zinc (Johnson et al. 1988; Spencer et al. 1985).

Absorption of zinc occurs from all segments of the intestine, although the largest proportion of zinc absorption occurs from the duodenum (Methfessel and Spencer 1973). The zinc absorption process includes both passive diffusion and a carrier-mediated process (Tacnet et al. 1990). The intestinal absorption of zinc appears to be a saturable carrier-mediated process at low zinc dose levels involving a cysteine-rich intestinal protein (CRIP) (Davies 1980; Gunshin et al. 1991; Hempe and Cousins 1992; Sturniolo et al. 1991). This protein binds zinc entering the intestinal cells from the lumen (Hempe and Cousins 1991). CRIP has a limited binding capacity for zinc

and becomes saturated when zinc concentration in the intestine is high. Metallothionein, a metal binding protein may contribute to zinc homeostasis at higher zinc absorption. Like several other metals, zinc can induce metallothionein production in intestinal mucosal cells (Richards and Cousins 1975). Zinc binds to metallothionein, which remains in the mucosal cells lining the gastrointestinal tract, and the bound metal is excreted from the body upon sloughing off of these cells. Although the affinity of zinc for metallothionein is relatively low, the protein may thus serve to prevent absorption of excess zinc in the body (Foulkes and McMullen 1987). Thus, absorption of zinc in rats is increased when metallothionein levels are lower (Flanagan et al. 1983). It is hypothesized that zinc entering luminal cells is associated with CRIP, and a small amount is bound to metallothionein; however, as the luminal zinc concentration increases, the proportion of cytosolic zinc associated with CRIP is decreased with a concomitant increase in zinc binding to metallothionein (Hempe and Cousins 1992). Further details on the influence of CRIP and metallothionein on zinc absorption are provided in Section 2.3.5, Mechanisms of Action.

Phytate and high phosphorus intakes in animals decrease zinc absorption. In humans, dairy products that contain both calcium and phosphorus decrease zinc absorption and plasma zinc concentration (Pecoud et al. 1975). Zinc binds to phosphate which results in coprecipitation of zinc with calcium phosphate in the intestines (Nelson et al. 1985). Dietary phytate also reduces zinc absorption. The addition of 400 pmol phytate to the diet decreased zinc absorption from 43.3±17.9% in females fed bread containing 0.02 mg zinc/kg (zinc-65 isotope) to 14.3±3.2% (Sandstrom and Sandberg 1992). Rats given diets supplemented with radiolabeled zinc and phytate excreted significantly more zinc in the feces than rats given diets supplemented with radiolabeled zinc but without phytate (Davies and Nightingale 1975). The study authors suggested that the decrease in absorption was due to the formation of zinc-phytate complexes in the intestines. Phytate also reduced reabsorption of zinc secreted into the gastrointestinal tract of humans (Sandstrom and Sandberg 1992).

Endogenous substances, such as amino acids, can influence the absorption of zinc. Complexing of zinc with amino acids generally enhances its absorption in all segments of the intestine (Wapnir and Stiel 1986). Although neither zinc nor the amino acid proline are readily absorbed in the colon, complexing of zinc with proline during an *in vivo* intestinal perfusion in rats resulted in increased zinc absorption.

Acrodermatitis enteropathica is a metabolic disorder that results in the malabsorption of zinc. However, when patients afflicted with this disorder were treated with human milk, zinc absorption was enhanced (Lombeck et al. 1975). It was reported by Evans (1980) that patients with acrodermatitis enteropathica have an impaired tryptophan metabolic pathway. Picolinic acid, a chief metabolite of tryptophan, is also a constituent of human milk. Picolinic acid is secreted by the pancreas into the intestinal lumen. A study by Boosalis et al. (1983) demonstrated that patients with pancreatic insufficiency had difficulty absorbing zinc administered as zinc sulfate. However, when these pancreatic-insufficient patients were given zinc as zinc picolinate, the extent of zinc absorption was similar to that of healthy controls. Zinc absorption may depend on the bioavailability of picolinic acid. Such a mandatory role of picolinic acid in absorption has not been confirmed (Bonewitz et al. 1982).

The addition of prostaglandin E, (PGE,) to the mucosal media of everted jejunal sacs from rats significantly increased zinc transport (Song and Adham 1979). In contrast, similar addition of prostaglandin F_2 (PGF_2) significantly decreased zinc transport. Addition of PGF_2 to the serosal side of the jejunal sacs increased the transport of zinc to the mucosal side; PGE_2 decreased the serosal to mucosal transport of zinc. The mechanism by which prostaglandins regulate zinc transport has not been established (Song et al. 1992). The limitation of the *in vitro* study is the absence of vascular perfusion and consequent trapping of metals in the submucosal tissue. Hence, studies of absorption of heavy metals, including zinc, in everted sacs have limited physiological relevance (Foulkes 1984) but may provide information useful for the design of future *in vivo* experiments.

The presence of other trace metals (e.g., mercury, cadmium, copper) may also diminish zinc transport. Section 2.6 provides detailed information on the interaction of zinc with other metals.

2.3.1.3 Dermal Exposure

Dermal absorption of zinc occurs, but its mechanism is not clearly defined. Studies are very limited regarding the absorption of zinc through the skin. Historically, zinc oxide has been used clinically to promote the healing of burns and wounds (Gordon et al. 1981). Absorption has been observed in burn patients treated with gauze dressings containing zinc oxide (Hallmans 1977)

The pH of the skin, the amount of zinc applied, and the vehicle administered with zinc all affect the absorption of zinc (Agren 1990, 1991).

Zinc chloride was also absorbed through the intact skin of the rat (Keen and Hurley 1977). Absorption of zinc sulfate was greater than zinc oxide following 4-48-hour dermal application to open wounds in Sprague-Dawley rats (Agren et al. 1991). About 12% of zinc oxide (0.25 mg zinc/cm^2) from the dressing reached the wound while 65% of zinc sulfate (0.066 mg zinc/cm^2) reached the wound. The data suggest that zinc oxide applied to wounds resulted in sustained delivery of zinc ions causing constant wound-tissue zinc levels. In contrast, zinc sulfate, being more water soluble than zinc oxide, is rapidly transferred into the blood and, therefore, caused decreased wound-tissue zinc levels (Agren et al. 1991).

2.3.2 Distribution

Zinc is one of the most abundant trace metals in humans. It is found normally in all tissues and tissue fluids and is a cofactor in over 200 enzyme systems. Together, muscle and bone contain approximately 90% of the total amount of zinc in the body (≈60% and 30%, respectively) (Wastney et al. 1986). Organs containing sizable concentrations of zinc are the liver, gastrointestinal tract, kidney, skin, lung, brain, heart, and pancreas (Bentley and Gribb 1991; Drinker and Drinker 1928; He et al. 1991; Llobet et al. 1988a). High concentrations of zinc were also detected in the prostate (Forssen 1972) retina, and sperm (Bentley and Grubb 1991). Zinc levels may vary considerably from one individual to another (Forssen 1972).

To some degree, the distribution of zinc in some tissues appears to be regulated by age (Schroeder et al. 1967). Zinc concentrations increase in the liver, pancreas, and prostate and decrease in the uterus and aorta with age. Levels in the kidneys and heart peak at approximately 40-50 years of age and then decline.

Zinc is present in blood plasma, erythrocytes, leukocytes, and platelets, but is chiefly localized within erythrocytes (of which 87% is in carbonic anhydrase, the major binding site) (Ohno et al. 1985). Zinc deficiency has been demonstrated to decrease the ability of erythrocytes to resist hemolysis *in vitro*. This finding suggests that zinc stabilizes the erythrocyte membrane. In plasma, two-thirds of the zinc is bound to albumin; the remainder is bound primarily to α_2-macroglobulin

(Bentley and Grubb 1991; Giroux et al. 1976; Wastney et al. 1986). It appears that the limited number of binding sites for zinc in plasma albumin and macroglobulin regulates the amount of zinc retained by the body (Andermann and Dietz 1982). Albumin-bound zinc has been correlated with plasma zinc levels, whereas α_2-macroglobulin shows no correlation with plasma zinc levels.

Hormones, such as the adrenocorticotrophic hormone (ACTH), appear to regulate the concentration of zinc in the liver. ACTH, secreted by the anterior pituitary gland, stimulates the secretion of glucocorticoids. Glucocorticoids, or hormones with glucocorticoid activity, have been shown *in vitro* to stimulate the net zinc uptake in cultured liver cells and at the same time activate the gene that regulates metallothionein synthesis (Failla and Cousins 1978). However, there are no *in vivo* data to support these *in vitro* findings. Metallothionein in the cells of the intestinal mucosa binds zinc, thus regulating its release into the blood.

The transfer of zinc across perfused placentas is slow; only ≈3% of maternal zinc reached the fetal compartment in 2 hours (Beer et al. 1992). The *in vitro* transfer of zinc between mother and fetus is bidirectional, with binding in the placenta (Beer et al. 1992). It is proposed that zinc uptake in the placenta involves a potassium/zinc transport system (Aslam and McArdle 1992). Newborns may also be exposed to zinc from their mothers by milk transfer of zinc during lactation (Rossowska and Nakamoto 1992).

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans after inhalation exposure to zinc. However, occupational studies provided indirect evidence that zinc may distribute to tissues to produce systemic effects (Brown 1988; Drinker et al. 1927a; Malo et al. 1990; McCord et al. 1926; Rohrs 1957; Sturgis et al. 1972).

Zinc levels in the lungs of cats peaked immediately after acute exposure to 12-61 mg zinc/kg/day as zinc oxide for approximately 3 hours and remained high for 2 days postexposure, then dropped significantly thereafter (Drinker and Drinker 1928). Levels in pancreas, liver, and kidneys increased slowly.

2.3.2.2 Oral Exposure

A single oral dose of 0.7 mg zinc/kg as zinc sulfate given to I1 individuals resulted in peak zinc levels in the plasma at 2-3 hours (Statter et al. l988; Sturniolo et al. 1991). Similarly, Neve et al. (1991) reported peak serum zinc concentration at 2.3 hours with 0.7 mg zinc/kg as zinc sulfate.

Following feeding of 191 mg zinc/kg/day as zinc acetate to rats for 3 months, increased zinc levels were significant in the heart, spleen, kidneys, liver, bone, and blood (Llobet et al. 1988a). The greatest increases were in bone (258%) and blood (520%). Elevated zinc levels were found in the kidneys and liver of mice fed 76.9 mg zinc/kg/day as zinc sulfate (Schiffer et al, 1991) or 38 mg zinc/kg/day as zinc nitrate (Cooke et al. 1990) for approximately 1 month. The kidneys and pancreas had higher zinc levels than the liver and carcass of rats fed diets containing 1.1 mg/kg/day for an unspecified duration (Weigand and Kirchgessner 1992). Newborn, young, and adult mice receiving a single oral dose of 4.6 mg zinc/kg as zinc chloride generally had the highest levels of zinc in the liver, kidneys, lungs, bone, muscle, and carcass 1 day after dosing (He et al. 1991). However, the amount of zinc in the lungs, muscle, and femur decreased with age.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to zinc. Animal data on the distribution of zinc following dermal exposure are limited. Elevated serum zinc levels occurred with the application of zinc oxide or zinc sulfate to skin wounds of Sprague-Dawley rats for 4-48 hours (Agren et al. 1991). Serum zinc level peaked at 4 hours in rats treated with zinc sulfate, while levels were slightly elevated for 48 hours in rats administered zinc oxide. The differences may be attributed to the absorbability of the zinc compounds.

2.3.3 Metabolism

Plasma provides a metabolically active transport compartment for zinc (Cousins 198.5). Zinc is most often complexed to organic ligands (existing in loosely or firmly bound fractions) rather than free in solution as metallic ion (Gordon et al. 1981). Zinc is found in diffusible or nondiffusible forms in the blood (NAS/NRC 1979). In the diffusible form, approximately two-thirds of plasma

zinc is freely exchangeable and loosely bound to albumin (Cousins 1985); the zinc-albumin complex has an association constant of about 10^6 (NAS/NRC 1979). The diffusible form of zinc also includes zinc bound to amino acids (primarily histidine and cysteine). The zinc-albumin complex is in equilibrium with the zinc-amino acid complex (Henkin 1974). The zinc-amino acid complex can be transported passively across tissue membranes to bind to proteins. An important binding protein in the kidney and liver is metallothionein, although other tissue-binding proteins may be present.

In the nondiffusible form, a small amount of circulating zinc is tightly bound to α_2-macroglobulin in the plasma (Cousins 1985). Zinc is incorporated into and dissociated from α_2-macroglobulin only in the liver (Henkin 1974). This zinc-protein complex has an association constant of $>10^{10}$ (Henkin 1974; NAS/NRC 1979). Th e zinc bound to α_2-macroglobulin is not freely exchangeable with other zinc ligands (i.e., zinc-albumin and zinc-amino acid complexes) in serum.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Information was limited regarding zinc excretion following inhalation exposure in humans. Workers exposed to zinc oxide fumes had elevated levels of zinc in the urine (Hamdi 1969) indicating that this is a route of excretion.

No studies were located regarding excretion in animals after inhalation exposure to zinc.

2.3.4.2 Oral Exposure

The principal route of excretion of ingested zinc in humans is through the intestine (Davies and Nightingale 1975; Reinhold et al. 1991; Wastney et al. 1986). Zinc loss in the body is by secretion via the gut, and the remainder occurs in the urine (Wastney et al. 1986). Fecal excretion of zinc increases as intake increases (Spencer et al. 1985). Excretion of zinc in the urine also reflects zinc intake (Wastney et al. 1986). Minor routes of elimination are saliva secretion, hair loss, and sweat (Greger and Sickles 1979; Hambidge et al. 1972; Henkin et al. 1975a; Prasad et al. 1963a;

There was a linear increase in fecal excretion of zinc in proportion to dietary intake in rats fed supplementations of 32 mg zinc/kg/day as zinc oxide for 7-42 days (Ansari et al. 1975) or 50-339 mg/kg/day for 21 days (Ansari et al. 1976). No differences in fecal excretion, total excretion, or retention of zinc were found among rats given diets containing different forms of zinc (Seal and Heaton 1983). Rats receiving 2.65 mg zinc/kg/day as zinc chloride, zinc sulfate, zinc phosphate, or zinc citrate, over a 4-day period excreted 87-98% of intake.

A study by Alexander et al. (1981) demonstrated that zinc is excreted in the bile of rats. Analysis of the bile indicated that the zinc is primarily complexed with reduced glutathione. Treatment of these rats with diethylmaleate, which conjugates with reduced glutathione and restricts its availability, depressed the biliary excretion of zinc. This depression confirms a relationship between zinc and glutathione and suggests that zinc is transferred from liver to bile by a glutathione-dependent process.

Other factors may affect zinc excretion. For example, low dietary intake of zinc or malnutrition can increase the urinary excretion of zinc. This release of zinc is a result of tissue breakdown and catabolism during starvation; and elevated urinary excretion of zinc may persist after intake levels return to normal (Spencer et al. 1976). Administration of histidine or high-protein diet may increase urinary zinc excretion; however, a corresponding increase in zinc absorption may maintain zinc balance in the body (Henkin et al. 1975b; Hunt et al. 1991).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to zinc.

2.3.5 Mechanisms of Action

The absorption of zinc from the intestine is homeostatically controlled. A study by Hempe and Cousins (1992) found that CRIP, a diffusible intracellular zinc carrier, binds zinc in the mucosa during absorption; this process appears to be saturable (Gunshin et al. 1991; Hempe and Cousins 1992; Sturniolo et al. 1991). Zinc transport in the intestinal lumen is also influenced by metallothionein which can inhibit zinc absorption by competing with CRIP for zinc (Hempe and Cousins 1992). CRIP binds about 40% of radiolabeled zinc entering the intestinal cells from the

lumen in ligated loops of the small intestine of anesthetized rats when the zinc concentration is low (5 μM), but only 14% of the radiolabel when the concentration is high (300 μM) (Hempe and Cousins 1991). These findings suggest that CRIP has a limited binding capacity for zinc and becomes saturated when zinc concentration in the intestine is high (Hempe and Cousins 1992).

High luminal zinc concentrations may damage the brush border membrane, allowing zinc to enter the cell and bind nonspecifically to cell proteins and other ligands (Cousins 1985; Hempe and Cousins 1992). Within the intestinal lumen, a number of factors appears to influence the availability of zinc for absorption. Methionine, histidine, cysteine, reduced glutathione, citrate, and prostaglandin E_2 increase the intestinal uptake of zinc (Song et al. 1992) whereas inorganic inhibitors of zinc absorption include cadmium, copper, calcium, and ferrous iron (Hamilton et al. 1978; Harford and Sarkar 1991; Ogiso et al. 1979; Spencer et al. 1992; Yoshida et al. 1993). The mechanism of inhibition has not been clearly elucidated, but it is believed to involve competition for zinc binding sites in the intestinal mucosal cells; an effect on charge distribution on the mucosal membrane has also been suggested (Foulkes 1985). The organic inhibitors, including phytate and some components of dietary fiber, are believed to complex with zinc and decrease its availability. In the mucosal cell, zinc is associated with metalloproteins, including metallothionein. The release of zinc from the intracellular protein ligands and its transfer to the blood may involve diffusion of complexes with glutathione and similar compounds (Foulkes 1993).

In the plasma, albumin is the primary carrier for zinc, with smaller amounts of zinc bound to α_2-macroglobulin and amino acids (Giroux et al. 1976). The albumin-bound zinc represents the metabolically active pool of zinc. Zinc is loosely bound in plasma, and albumin-bound zinc can readily be given up to tissues; however, the mechanisms are not fully elucidated. Zinc is initially concentrated in the liver after ingestion, and is subsequently distributed throughout the body. The liver, pancreas, bone, kidney, and muscle are the major tissue storage sites. When plasma zinc levels are high, liver metallothionein synthesis is stimulated, which facilitates the retention of zinc by hepatocytes (Richards and Cousins 1975). A storage form of zinc has not been identified in soft tissues, with the possible exception of zinc metallothionein. Zinc in bone is relatively unavailable for use by other tissues.

Metal fume fever is the primary effect observed in workers exposed to zinc oxide fumes or dust (Blanc et al. 1991; Brown 1988; Drinker et al. 1927b; Vogelmeier et al. 1987). Metal fume fever

usually occurs 3-10 hours after exposure, and the symptoms persist for 24-48 hours. The exact pathogenesis of metal fume fever is not known It is believed to be an immune response to the inhaled zinc oxide (Mueller and Seger 1985). It has been suggested that the zinc oxide causes inflammation of the respiratory tract and the release of histamine or histamine-like substances. In response, an allergen-antibody complex is formed that may elicit an allergic reaction upon subsequent exposure to the allergen. In response to the allergen-antibody complex, an antiantibody is formed. The anti-antibody dominates with continued exposure to the zinc oxide, thereby producing a tolerance. When the exposure is interrupted and re-exposure occurs, the allergen-antibody complex dominates, producing an allergic reaction and symptoms of metal fume fever (McCord 1960).

Oral exposure to high levels of zinc has caused anemia, decreased levels of HDL cholesterol, and pancreatic damage in humans (Black et al. 1988; Chandra 1984; Chobanian 1981; Hooper et al. 1980; Murphy 1970) and animals (Allen et al. 1983; Aughey et al. 1977; Drinker et al. 1927d; Katya-Katya et al. 1984; Klevay and Hyg 1973; Maita et al. 1981; Straube et al. 1980). The mechanisms involved in the pancreatic damage have not been elucidated. The anemia and possibly the decreased HDL cholesterol levels are thought to be caused by a zinc-induced copper deficiency. Although it is generally accepted that the anemia is the result of copper deficiency, the relationship between zinc and copper levels and HDL cholesterol levels has been extensively debated (Fischer et al. 1980; Katya-Katya et al. 1984; Klevay and Hyg 1973; Murthy and Petering 1976) and is beyond the scope of this profile.

2.4 RELEVANCE TO PUBLIC HEALTH

Zinc is required for normal growth, bone formation, brain development, behavioral response, reproduction, fetal development, sensory function (taste and smell), immune function, membrane stability, and wound healing. The recommended dietary allowance for zinc is 5 mg/day for infants (0-l year), 10 mg/day for children (l-10 years), 15 mg/day for males (11-15 + years), 12 mg/day for females (11-15 + years), 15 mg/day for pregnant women, 19 mg/day during the first 6 months of lactation, and 16 mg/day during the next 6 months of lactation (NAS/NRC 1989b). There are over 100 enzymes that require zinc for maximum catalytic activity (Cousins 1985). Zinc provides structural integrity to the enzyme and/or participates directly in catalysis. Not all of the zinc enzymes are responsive to changes in zinc intake (Kirchgessner et al. 1976). Decreases in plasma

alkaline phosphatase, alcohol dehydrogenase, connective tissue thymidine kinase, pancreatic carboxypeptidase A, and liver nuclear DNA-dependent RNA polymerase have been observed during deficiency. No changes in carbonic anhydrase, lactate dehydrogenase, or malate dehydrogenase activities were observed.

Zinc deficiency has been observed in the inhabitants of Middle Eastern countries who have diets rich in cereals that provide little readily available zinc and who have a high incidence of parasitic infections, geophagia, and chronic infectious diseases (Prasad et al. 1963a, 1963b). The primary effects observed in males were growth failure, delayed sexual maturation, and iron-deficiency anemia (Prasad et al. 1963b). Marginal zinc deficiency has been observed in the United States (Hambidge et al. 1972; Henkin et al. 1976). Signs of deficiency include poor growth, anorexia, hypogeusia, and dysgeusia. Supplementation with zinc reversed these signs. Impaired fetal development, delayed wound healing, oligospermia, decreased serum testosterone concentration, hyperammonemia, impaired immune function, psoriasis, and decreased lean body mass have been observed in humans with experimentally induced marginal zinc deficiency (Prasad 1991) or with secondary zinc deficiency due to malabsorption, sickle cell anemia (Cunningham-Rundles et al. 1990) or cirrhosis of the liver (Parodi et al. 1991).

Metal fume fever has been observed in humans who inhaled high concentrations of zinc oxide fumes. Metal fume fever is believed to be an immune response characterized by increased body temperature, impaired lung function, increased number of leukocytes in the blood, and bronchoalveolar lavage fluid. Similar effects were observed in animals. Metal fume fever has been observed after acute, intermediate, and chronic inhalation exposures to zinc oxide.

The principal effects observed in humans after oral acute doses of zinc oxide include abdominal pain, vomiting, anemia, and pancreatic damage. The dose associated with these effects is >10-fold higher than the RDA for zinc. Similar effects are observed in animals. Kidney damage has also been observed in animals following intermediate exposure, but not in humans.

There is limited information on the dermal toxicity of zinc. Dermal application of zinc acetate, zinc chloride, or zinc sulfate caused slight-to-severe skin irritation in rabbits, guinea pigs, and mice. These compounds would also be irritating to human skin. In contrast, dermal exposure to zinc oxide did not usually cause skin irritation to humans and animals. However, a few workers

who routinely became covered with zinc oxide dust have had pustules on the axilla and inner thighs possibly formed in response to plugging of glands by sweat, bacteria, and zinc oxide dust.

Four cases of premature deliveries and stillborns were reported following supplemental oral doses of 0.6 mg zinc/kg/day during the last trimester of pregnancy in humans. Increased fetal resorptions and stillbirths were observed in rats fed diets containing ≥200 mg zinc/kg/day. Rats failed to reproduce after being fed 250 mg zinc/kg/day for 5 months. It is not known if this would also occur in humans. Positive genotoxic results have been observed *in vivo* and *in vitro* in mammalian cells. There are limited human and animal data suggesting that zinc is not carcinogenic, but more information is needed to further assess this inference.

Following ingestion of large amounts of zinc, increased levels are found in the heart, spleen, kidneys, liver, bone, and blood. It is not known if there is a relationship between the toxic effects observed in humans and tissue storage levels of zinc. Zinc stored in bone is not readily available to the general metabolic pool. During decreased calcium intake or bone resorption, zinc is released from the bone. It is not known if there are any toxic effects associated with this release of zinc.

The general population is primarily exposed to zinc by ingestion. Several factors influence the potential for adverse health effects after oral exposure to zinc, including age, gender, and diet. It is possible that people living near a hazardous waste site would be exposed to increased levels of zinc in the drinking water. This, in addition to the zinc naturally occurring in food, may result in toxic effects. Inhalation exposure to the general population is less likely because ambient air levels of zinc are generally very low; however, near a smelter, levels can be as high as 5 $\mu g/m^3$. Zinc dust from contaminated soil may also be inhaled. It is not likely that exposure to this level of airborne zinc would result in toxic effects. Dermal exposure may occur from skin contact with contaminated soil containing zinc.

Minimal Risk Levels for Zinc

Inhalation MRL's

No inhalation MRLs have been derived for zinc (see Table 2-l and Figure 2-l). A number of acute-duration human studies have identified metal fume fever as an end point of concern. Animal studies corroborate the effects observed in humans; however, other possible targets of toxicity were not examined. Only one intermediate-duration inhalation study in humans was located. In this study, no exposure levels were reported; thus, the study could not be used as the basis for the derivation of an intermediate-duration MRL. No exposure-related effects on lung function were observed in a group of welders chronically exposed to zinc; however, the exposure level was not reported. Thus, no chronic-duration inhalation MRL could be derived.

Oral MRLs

- An MRL of 0.3 mg zinc/kg/day has been derived for intermediate oral exposure to zinc. The MRL was based on hematological effects, specifically decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase activity, in women given daily supplements of 50 mg zinc as zinc gluconate for 10 weeks (Yadrick et al. 1989). The LOAEL of 1 mg/kg/day was derived from 9.72 mg zinc/day, the estimation of dietary zinc for females (20-30 years old) from the FDA Total Diet study for 1982-1986 (Pennington et al. 1989) plus 50 mg zinc/day, the reported supplemental zinc dose. A reduction in erythrocyte superoxide dismutase activity was also seen in males receiving daily zinc supplements of 50 mg for 6 weeks (Fisher et al. 1984). Zinc supplementation has been shown to decrease HDL levels with daily doses of at least 50 mg zinc for 12 weeks (Black et al. 1988; Chandra et al. 1984; Hooper et al. 1980). These studies suggest that there is a dose-response trend and that 50 mg/day is the threshold LOAEL for zinc. In animals, decreased hematocrit has been observed at higher doses (Jenkins and Hidiroglou 1991; Maita et al. 1981; Smith and Larson 1946).

- The intermediate oral MRL of 0.3 mg zinc/kg/day has been adopted as the chronic oral MRL. The chronic oral MRL is expected to be without adverse effects when consumed on a daily basis over a long period of time; neither inducing nutritional deficiency in healthy,

non-pregnant, adult humans ingesting the average American diet nor causing undesirable inhibition of normal lipid transport. The MRL was not based on a chronic-duration oral study due to a lack of adequate long-term studies in humans and animals. The chronic human study by Hale et al. (1988) provides support for the Yadrick et al. (1989) study and suggests that hematological effects may occur at higher zinc doses. A significant decrease in red blood cells in females receiving daily supplements of 2 mg zinc/kg/day for an average of 8 years was reported by Hale et al. (1988). Furthermore, decreases in serum creatinine, total protein, and uric acid and an increase in mean MCH were observed in the treated male and female subjects (mean age of 78 years) compared to controls.

In general, the MRL is an estimate of the daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. The intermediate and chronic oral MRLs for zinc do not provide levels of concern for zinc in infants, children, or lactating women. Also, the MRLs are based on soluble zinc salts, and it is less likely that nonsoluble zinc compounds would have these effects at this level.

No oral acute MRL was derived for zinc (see Table 2-2 and Figure 2-2). A number of case reports involving acute exposure were located. These reports suggest that the gastrointestinal tract and the pancreas are end points of concern, and that the adrenal cortex may also be affected. A great deal of uncertainty exists regarding the exposure levels for these studies. An oral exposure study in sheep was also identified. Because sheep are ruminants, it is doubtful that they are a good model for human toxicity.

Death. Death from respiratory failure has occurred in humans after acute inhalation exposure to zinc chloride smoke (Evans 1945; Hjortso et al. 1988; Milliken et al. 1963). However, the level of airborne zinc was not determined. Furthermore, exposure to zinc was accompanied by exposure to other chemicals. Hence, death could not be attributed exclusively to zinc exposure.

Only limited information was located regarding death in animals from inhalation exposure to zinc. The LCT_{50} of zinc chloride was reported as 11,800 mg-min/m^3 (Schenker et al. 1981), but the basis for this value was not provided. Exposure to 119.3-121.7 mg zinc/m^3 as zinc chloride smoke for 3-20 weeks decreased the survival of guinea pigs and mice (Man-s et al. 1988). Death was

reported in ferrets orally exposed to 390 mg zinc/kg/day as zinc oxide (Straube et al. 1980) and mice exposed to 1,110 mg zinc/kg/day as zinc sulfate (Maita et al. 1981) following acute and intermediate oral exposures, respectively. Adverse systemic effects were observed in these animals, but the specific cause of death could not be determined. Acute oral LD50 values in rats depend on the specific zinc compound and range from 237 mg zinc/kg as zinc acetate to 623 mg zinc/kg as zinc sulfate. In mice, the range of LD_{50} values is 86 mg zinc/kg as zinc acetate to 605 mg zinc/kg as zinc chloride (Domingo et al. 1988a). Death from exposure to environmental levels of zinc is improbable.

Systemic Effects

Respiratory Effects. Respiratory disorders have been observed in humans and animals after acute inhalation exposure to zinc and its compounds. No adverse respiratory effects have been observed following ingestion or dermal application of zinc or its compounds.

Acute exposure to high concentrations of airborne zinc oxide in humans causes metal fume fever. Zinc oxide penetrates the alveoli, damages the lung tissue, and transiently impairs pulmonary function (Blanc et al. 1991; Brown 1988; Drinker et al. 1927b; Vogelmeier et al. 1987). Lung volumes are decreased, as is the carbon monoxide diffusion capacity (Drinker et al. 1927b; Mueller and Seger 1985; Sturgis et al. 1927). Metal fume fever is believed to be the result of an immune reaction to inhaled metal oxide particles (Mueller and Seger 1985).

Respiratory tract irritation occurs in both humans and animals (Drinker and Drinker 1928; Sturgis et al. 1927) after exposure to zinc oxide. Most laboratory animals, except guinea pigs, begin to present respiratory abnormalities (e.g., pulmonary congestion, peribronchial leukocytic infiltration) at exposure levels similar to those that cause effects in humans (Drinker and Drinker 1928). Cats exhibited more severe effects, including bronchopneumonia, than other animals (Drinker and Drinker 1928).

In humans, inhalation of zinc chloride causes greater damage to respiratory tissue than inhalation of zinc oxide. Reported lesions included acute pneumonitis, ulceration of mucous membranes, subpleural hemorrhage, and pulmonary fibrosis. Exposed individuals have died from respiratory

distress syndrome (Evans 1945; Hjortso et al. 1988; Johnson and Stonehill 1961; Matarese and Matthews 1966; Milliken et al. 1963; Schenker et al. 1981).

The studies in humans and animals reveal that inhalation of zinc as particulate or fume can result in respiratory ailments (Drinker and Drinker 1928; Sturgis et al. 1927). These zinc fumes or particles are a particular problem for industrial workers. It is possible that inhalation exposure to zinc compounds could result in respiratory effects in people living near a hazardous waste site.

Cardiovascular Effects. In humans, oral exposure of intermediate duration to zinc has decreased serum HDL cholesterol levels (Chandra 1984; Hooper et al. 1980). Although this is not a direct effect on the cardiovascular system, the decrease in HDL levels may be associated with an increased risk of coronary artery disease. However, another study showed no effect on HDL levels and a decrease in LDL levels (Samman and Roberts 19&S), which would be associated with a decreased risk of coronary artery disease. More information on this effect is presented later in this section under Other Systemic Effects. It is not known if exposure to zinc in air, water, or soil would result in cardiovascular effects in people living near hazardous waste sites.

Gastrointestinal Effects. Gastrointestinal irritation (abdominal pain, vomiting, nausea, esophageal erosions, and gastric hemorrhagic erosion) has been observed in humans after acute ingestion of zinc compounds. In most cases, the actual exposure levels associated with these effects are not known (Anonymous 1983; Chobanian 1981; Potter 1981). In a 15year-old girl, epigastric discomfort, gastritis, and hemorrhagic erosion were observed after exposure to 2.6 mg zinc/kg/day as zinc sulfate for 1 week (Moore 1978). Longer-term human oral studies found no signs of gastrointestinal irritation at dose levels of ≤4.3 mg zinc/kg/day (Chandra 1984; Hallbook and Lanner 1972; Hoffman et al. 1988; Hooper et al. 1980; Shah et al. 1988). Gastrointestinal effects were not investigated in human inhalation studies. One mechanism for clearing particles (≥3 pm in diameter) from the respiratory tract is swallowing. It is possible, therefore, that exposure to airborne zinc would result in gastrointestinal effects. Ulceration of the forestomach and intestinal hemorrhages have been observed in mice ingesting 1,110 mg zinc/kg/day as zinc sulfate and ferrets consuming 390 mg zinc/kg/day as zinc oxide, respectively (Maita et al. 1981; Straube et al. 1980). Gastrointestinal irritation could occur in humans exposed to zinc in air, water, or soil near hazardous waste sites.

Hematological Effect. In humans, oral chronic exposure to high levels of zinc caused decreased levels of hemoglobin and hematocrit, and/or microcytic anemia (Broun et al. 1990; Hale et al. 1988; Hoffman et al. 1988; Patterson et al. 1985; Porter et al. 1977; Prasad et al. 1978). Reduction in serum ferritin and erythrocyte superoxide dismutase activity in humans have also been reported in intermediate-duration studies (Fischer et al. 1984; Yadrick et al. 1989). Similar effects have been observed in rats (Maita et al. 1981), mice (Maita et al. 1981; Walters and Roe 1965), rabbits (Bentley and Grubb 1991), dogs (Drinker et al. 1927d), ferrets (Straube et al. 1980), and preruminant calves (Jenkins and Hidiroglou 1991). The anemia is believed to be the result of zinc-induced copper deficiency. *De novo* synthesis of metallothionein in the intestinal mucosal cells is induced by high levels of dietary zinc (Cousins 1985). Copper has a higher binding affinity than zinc to metallothionein and will replace the zinc. Metallothionein-bound copper is not transferred to the portal blood and is excreted in the feces when the intestinal mucosal cell is sloughed off (Fischer et al. 1981; L'Abbe and Fischer 1984a). Decreases in hemoglobin and hematocrit levels were larger in animals fed diets low in copper and high in zinc, compared to those fed a diet with adequate levels of zinc but low levels of copper, or those fed a diet with excess zinc but adequate levels of copper (Johnson and Flagg 1986). Although the interaction between copper and zinc has been well established in animals, conflicting results have been observed in human studies. Copper deficiency has been diagnosed in individuals chronically (14-24 months) taking zinc sulfate for the treatment of sickle-cell anemia or celiac disease (Porter et al. 1977; Prasad et al. 1978). Studies have also reported increases in fecal excretion of copper and decreased copper retention in healthy subjects consuming high levels of zinc (Burke et al. 1981; Festa et al. 1985; Greger et al. 1978a). Other studies in humans have not reported altered copper metabolism (Colin et al. 1983; Greger et al. 1978b; Henkin et al. 1976; Samman and Roberts 1987; Taper et al. 1980). Anemia might occur in humans if exposed orally to zinc found near hazardous waste sites.

Leukocytosis has been reported in a number of studies of acute human exposure to zinc oxide fumes (Brown 1988; Drinker et al. 1927a; Malo et al. 1990; Mueller and Seger 1985; Rohrs 1957; Sturgis et al. 1927). Data are contradictory regarding whether inhalation exposure of humans to zinc oxide can result in anemia (Hamdi 1969; McCord et al. 1926), and very limited human data suggest that dermal exposure to zinc chloride perhaps could cause anemia (DuBray 1937). None of the animal inhalation or dermal exposure studies examined hematological parameters. Altered copper metabolism has not been observed following subcutaneous administration of zinc in rats

(Hill et al. 1984). The limited data suggest that anemia might occur in humans exposed to zinc by inhalation or dermally near hazardous waste sites.

Musculoskeletal Effects. Musculoskeletal effects have not been observed in humans or animals after inhalation, oral, or dermal exposure to zinc or its compounds. Muscles and bones contain the highest levels of zinc, and bones are believed to serve as storage depots for zinc. It is not known whether high levels of zinc in bone might be a problem because of replacement of other minerals in the bone.

Hepatic Effects. Hepatic effects have not been observed in humans after inhalation, oral, or dermal exposure to zinc. Hepatic necrosis was observed in sheep after acute and intermediate exposures to zinc compounds (Allen et al. 1983). Intermediate oral exposure of rats to zinc compounds induced liver enzymes at a dose similar to that administered to sheep (Kadiiska et al. 1985). No histopathological alterations have been observed in the livers of rats (Drinker et al. 1927c; Kadiiska et al. 1985; Llobet et al. 1988a) or mink (Aulerich et al. 1991) after intermediateduration ral exposure to zinc. The weight of evidence suggests that the liver is not a primary target organ in humans or animals for zinc toxicity.

Renal Effects. No adverse renal effects have been observed in humans after inhalation or oral exposure to zinc compounds. Following intermediate oral exposure to zinc compounds, cellular damage in the glomerulus and proximal convoluted tubules and regressive lesions were observed in rats and mice (Llobet et al. 1988a; Maita et al. 1981). It is not known whether renal effects may be found in humans living near a hazardous waste site where zinc is present in the air, water, or soil.

Dermal/Ocular Effects. Adverse dermal or ocular effects have not been observed in humans after inhalation or oral exposure to zinc. When applied to the skin, zinc's effects differ with respect to the particular zinc salt. Zinc oxide creams are widely used to promote wound healing. No signs of skin irritation were observed in humans after dermal exposure to zinc oxide (Agren 1990). Intermediate to chronic exposure to high concentrations of zinc oxide dust has resulted in plugging and infection of the sebaceous glands on the axilla and inner thighs and in the pubic region (Batchelor et al. 1926; Turner 1921). It was reported that this effect was probably due to poor hygiene rather than the irritancy of the zinc oxide dust. In contrast, zinc chloride was

reported to be irritating to the eyes and skin at very high concentrations. Exposure to extremely high concentrations of zinc chloride-containing smoke resulted in reddened conjunctiva and running eyes in 34 persons, cornea1 burns in 2 persons, and small burns on the forehead or wrists of 4 persons (Evans 1945). In rabbits, guinea pigs, and mice, moderate-to-severe skin irritation was observed following dermal exposure to zinc acetate or zinc chloride. Slight irritation in mice, but no irritation in rabbits or guinea pigs, was observed after exposure to zinc sulfate; no signs of skin irritation were observed in rabbits, guinea pigs, or mice following application of zinc oxide (Lansdown 1991). Exposure to air or water at or near a hazardous waste site containing a sufficiently high concentration of one of the more caustic zinc salts could result in skin or eye irritation.

Other Systemic Effects. Elevated body temperature, a symptom of metal fume fever syndrome, was reported by workers inhaling zinc fumes and dust (Malo et al. 1990).

Systemic effects observed in humans and animals after oral exposure to zinc compounds include damage to the pancreas and adrenal gland, alterations in the serum lipid profile, and pituitary hyperactivity. An increase in serum amylase and lipase levels was observed in individuals ingesting single high-level doses of zinc (Chobanian 1981; Murphy 1970). These changes are indicative of pancreatic damage. Pancreatic lesions have been observed in rats (Maita et al. 1981) mice (Aughey et al. 1977; Maita et al. 1981) cats (Drinker et al. 1927d), ferrets (Straube et al. 1980) and sheep (Allen et al. 1983) after intermediate or chronic oral exposure to high levels of zinc compounds. Several of the intermediate-duration studies reported that the lesions were located in the acinar cells. Acinar cell necrosis and metaplasia in pancreas were reported in rats exposed to 565 mg/kg/day and mice exposed to 1110 mg/kg/day in an intermediate-duration study (Maita et al. 1981). The pancreatic acinar cells are responsible for the secretion of pancreatic lipase, pancreatic amylase, trypsinogen, chymotrypsinogen, and procarboxypeptidase. A chronic mouse study reported marked cellular alterations in the pancreatic islets during 12 months of oral exposure to 70 mg/kg/day (Aughey et al. 1977). The islets are involved in the secretion of insulin and glucagon. No changes in blood glucose or insulin levels and no change in the response to a glucose load were observed in humans after a single dose of 0.5 mg zinc/kg/day as zinc sulfate (Brandao-Neto et al. 1990b).

Decreases in serum cortisol levels in humans after a single oral dose of zinc sulfate may indicate adrenal cortical damage (Brandao-Neto et al. 1990a). A chronic-duration drinking water study in rats exposed to 70 mg/kg/day revealed hypertrophy and vacuolation of the zona fasciculata cells of the adrenal cortex (Aughey et al. 1977). Glucocorticoids (cortisol, cortisone, corticosterone, deoxycorticosterone) are secreted by cells in the zona fasciculata. It is not known if there is a relationship between the damage to the pancreatic islets and the hypertrophy of the zona fasciculata cells in the adrenal cortex.

Decreased levels of serum HDL cholesterol were observed in humans after oral exposure to high levels of zinc for an intermediate duration (Black et al. 1988; Chandra 1984; Hooper et al. 1980). No other consistent changes in the serum lipid profile (total cholesterol, LDL cholesterol, and triglyceride levels) were observed. Changes in HDL cholesterol were not observed in young subjects (mean age of 22 years) (Samman and Robetts 1988) or in elderly subjects (60-89-years old) who also received copper supplements for 3-12 months (Bogden et al. 1988). Increases in serum cholesterol have been observed in rats in some oral exposure studies (Katya-Katya et al. 1984; Klevay and Hyg 1973). However, other studies using similar zinc compounds, doses, and durations of exposure have not found alterations in the serum lipid profile of rats (Fischer et al. 1980; Woo et al. 1983). It is possible that inhalation, oral, or dermal exposure to zinc compounds at hazardous waste sites could cause pancreatic damage, adrenal gland damage, and an alteration in the serum lipid profile.

Immunological Effects. Although the pathogenesis of metal fume fever is uncertain, it is believed to be an immune response to submicron-sized particles of zinc oxide. In addition, increased levels of lymphocytes, polymorphonuclear leukocytes, and macrophages were observed in the bronchoalveolar lavage fluid of welders. Significant correlations between the concentration of airborne zinc and the levels of T cells were reported by Blanc et al. (1991). Impaired immune function was also observed in humans taking high-dose (4.3 mg zinc/kg/day) zinc sulfate supplements for 6 weeks (Chandra 1984). Immunotoxicity was not observed in mice after oral exposure to 76.9 mg zinc/kg/day as zinc sulfate for 4 weeks (Schiffer et al. 1991). Immune suppression was observed in rats given a single injection of zinc chloride (82 mg zinc/kg) (Yatsuyanagi et al. 1987). Because zinc particles flocculate in the atmosphere, it is unlikely that persons near hazardous waste sites would be exposed to zinc particles fine enough (≤1 μm in diameter) to enter the alveolar region of the lung and elicit an immune response. However,

ingestion of zinc in drinking water at concentrations found at hazardous waste sites could result in immune suppression.

Neurological Effects. Zinc appears to be necessary for normal brain function. Nonspecific signs and symptoms of neurotoxicity (headache, dizziness, lethargy, staggering gait) have been observed in humans after acute ingestion of large amounts of zinc compounds (Anonymous 1983; Murphy 1970; Potter 1981). Neurotoxicity has not been observed in animals after inhalation, oral, or dermal exposure to zinc or its compounds although minor neuron degeneration was observed in rats after a 10-day oral exposure (Kozik et al. 1988). *In vitro* investigations on the effects of zinc on neurological tissue indicate that zinc competitively inhibits the entry of calcium into nerve terminals, thereby influencing the release of neurotransmitters (Nishimura 1987). Zinc (at concentrations that may occur *in vivo*) is toxic to neurons and glial cells of the central nervous system *in vitro* cultures (Choi et al. 1988; Yokoyama et al. 1986). It is not known if neurological effects would occur in humans exposed to zinc in air, water, or soil at or near a hazardous waste site.

Reproductive Effects. Daily oral exposure to zinc sulfide to women during the last two trimesters did not cause any complications in pregnancies (Mahomed et al. 1989). No studies were located regarding reproductive toxicity in humans after inhalation or dermal exposure to high levels of zinc. Zinc deficiency in humans has been shown to result in abnormalities of labor, atonic bleeding, pre-term labor, and post-term pregnancies.

Oral exposure to high levels of zinc increased the incidence of pre-implantation loss in rats when exposure began on gestational day 0 and continued throughout the gestational period (Pal and Pal 1987). Failure to reproduce was observed in multiparous rats fed 250 mg zinc/kg/day as zinc carbonate for 150 days. In rats fed zinc for 21 days prior to mating, no reproductive effects were observed (Pal and Pal 1987). No histological alterations of the testes or ovaries were observed in rats and mice fed high levels of zinc in their diet (Maita et al. 1981). There are very limited data to suggest that reproductive effects might occur in humans exposed to zinc in air, water, or soil at or near a hazardous waste site.

Developmental Effects. There is only one report of developmental effects occurring in humans exposed to high levels of zinc compounds. In this brief report, three cases of stillbirths and one

case of premature delivery were observed in a group of women taking 0.6 mg zinc/kg/day as zinc sulfate supplements during the third trimester of pregnancy (Kumar 1976). Extreme caution must be taken in interpreting the results of this study. The number of subjects taking the supplements, demographic information on the women, and comparison of the exposed individuals to a unexposed control group were not reported. A number of other human studies found no developmental effects in the children of mothers taking zinc supplements (highest NOAEL is 0.3 g zinc/kg/day) (Kynast and Saling 1986; Mahomed et al. 1989; Simmer et al. 1991). Zinc deficiency in humans is associated with pregnancy complications, particularly growth retardation.

An increase in the incidence of fetal resorptions and decreased fetal weights were observed in rats after oral exposure to 200 mg zinc/kg/day prior to and during mating and throughout gestation (Schlicker and Cox 1968). Alopecia and hair discoloration were observed in the offspring of mice exposed to 260 mg zinc/kg/day (Mulhern et al. 1986). These effects are believed to be the result of zinc-induced copper deficiency. No developmental effects were observed in rats exposed to up to 100 mg zinc/kg/day (Schlicker and Cox 1968; Uriu-Hare et al. 1989).

Congenital malformations, such as exencephaly and rib fusions, have been observed in the offspring of pregnant golden hamsters injected intravenously with a single dose of 2 mg zinc sulfate/kg on day 8 of gestation (Ferm and Carpenter 1968). Similarly, single intraperitoneal injections of 12.5, 20.5, or 25 mg/kg zinc chloride on days 8, 9, 10, or 11 of gestation produced skeletal anomalies, including delayed ossification and rippled ribs without accompanying soft tissue defects in mice. Rippled ribs, an unusual anomaly, appeared when zinc salt was given on day 9 of gestation at a dose of 20.5 mg/kg, becoming more prevalent when 25 mg/kg of the salt was administered on day 11 of gestation (Chang et al. 1977). It is not known whether exposure to air, waker, or soil at or near a hazardous waste site could cause developmental effects in humans.

Genotoxic Effects. Genotoxicity studies conducted in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenic effects following zinc exposure.

Results of *in vivo* studies are shown in Table 2-4. A dominant lethal study in mice failed to show a mutagenic potential for zinc. However, chromosomal aberrations have been observed in bone

TABLE 2-4. Genotoxicity of Zinc *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian systems:			
Mouse	Dominant lethal	-	Vilkina et al. 1978
Mouse bone marrow	Chromosomal	+	DeKnudt and Gerber 1979
Mouse	Chromosomal	+	Voroshilin et al. 1978
Rat bone marrow	Chromosomal aberrations	+	Kowalska-Wachna 1988
Mouse bone marrow	Chromosomal aberrations	+	Gupta et al. 1991
Rat bone marrow	Sister chromatid exchange	+	Kowalska-Wachna 1988
Mouse	Micronucleus	-	Gocke et al. 1981
Drosophila	Sex-linked recessive lethal	-	Gocke et al. 1981

- = negative result; + = positive result

marrow cells following *in vivo* exposure to zinc (Vilkina et al. 1978). This effect was observed in rats exposed to 14.8 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 19&Q mice given intraperitoneal injections of 3.6 mg zinc/kg/day as zinc chloride (Gupta et al. 1991), and mice exposed to zinc oxide by inhalation (Voroshilin et al. .1978). Chromosomal aberrations caused by zinc were observed in the bone marrow cells of mice maintained on a low-calcium diet (Deknudt and Gerber 1979). Calcium may be displaced by zinc in calcium-depleted conditions, leading to chromosome breaks and/or interfering in the repair process (Deknudt and Gerber 1979). An increased incidence of sister chromatid exchange was observed in bone marrow cells of rats exposed to 17.5 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 1988).

Results of *in vitro* studies are shown in Table 2-5. Exposure to zinc as zinc sulfate or zinc chloride does not increase mutation frequencies in bacterial or mammalian cell culture test systems (Amacher and Paillet 1980; Gocke et al. 1981; Marzin and Vo Phi 1985; Nishioka 1975; Thompson et al. 1989; Venitt and Levy 1974; Wong 19&S). Similarly, there was no convincing evidence of a clastogenic effect in human lymphocytes exposed to 0.0003-0.00003 M zinc chloride (Deknudt and Deminatti 1978).

Cancer. No reliable human carcinogenicity data were located. The carcinogenicity of zinc following oral exposure has been evaluated in a study with mice (Walters and Roe 1965). In this study, mice were given 0, 190, or 951 mg zinc/kg/day as zinc sulfate in drinking water for 1 year. Relative to controls, no increase in tumor incidence was observed in treated mice. The investigation was not adequate for evaluating the carcinogenicity of zinc because of several study limitations (inadequate or lack of details of protocol, age, sex, number of animals tested, and purity of test material). Lung adenomas were not found in mice injected intraperitoneally with doses up to 5.3 mg/kg/day, three times a week, for 8 weeks (Stoner et al. 1976), but there was no indication that a sufficiently high dose was tested. Teratomas of the testes were observed in fowl given testicular injections of 2 mL of a 10% zinc sulfate solution (Falin and Gromzewa 1939) but they were not observed in rats given testicular injections of 23 mg zinc/kg/day as zinc sulfate (Guthrie 1956). The relevance of this study to public health is not known. EPA has determined that zinc is not classifiable as to its human carcinogenicity (IRIS 1993).

TABLE 2-5. Genotoxicity of Zinc *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (TA102)	Gene mutation	Not tested	-	Marzin and Vo Phi 1985
S. typhimurium (TA98, TA102, TA1535, TA1537)	Gene mutation	-(S9)	-	Wong et al. 1988
S. typhimurium (TA1538, TA98, TA100, TA1537)	Gene mutation	-(S9)	-	Thompson et al. 1989
S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutation	-(S9)	-	Gocke et al. 1981
Escherichia coli	Gene mutation	Not tested	-	Nishioka 1975
E. coli	Gene mutation	Not tested	-	Venitt and Levy 1974
Mammalian cells:				
Mouse lymphoma	Gene mutation	Not tested	-	Amacher and Paillet 1980
Mouse lymphoma	Gene mutation	+(S9)	+	Thompson et al. 1989
Human lymphocytes	Chromosomal aberrations	Not tested	+	Deknudt and Deminatti 1978
Chinese hamster ovary cells	Chromosomal aberrations	+(S9)	+	Thompson et al. 1989

- = negative result; + = positive result

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAWNRC 1989a).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989a). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to zinc are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989a). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by zinc are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an

intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Zinc

There is no simple measure of zinc body burden. Under normal physiological conditions, the plasma/serum zinc level is ≈1 μg/mL (NAS/NRC 1979) and the urinary level is 0.5 mg/g creatinine (Elinder 1986). S everal studies have reported increased levels of zinc in the serum and urine of humans and animals after inhalation, oral, or dermal exposure to zinc (Agren et al. 1991; Bentley and Grubb 1991; Brandao-Neto et al. 1990a; Hallmans 1977; Hamdi 1969; Keen and Hurley 1977; Neve et al. 1991; Statter et al. 19818; Sturniolo et al. 1991). However, relationships between serum and/or urine levels and zinc exposure levels have not been established.

Hair and nail samples provide a lasting record of long-term metal intake possibly over weeks or months (Hayashi et al. 1993; Wilhelm et al. 1991). Mean zinc concentrations of 129-179 μg/g have been estimated for nails (Hayashi et al. 1993; Wilhelm et al. 1991) and 102-258 μg/g for hair (Folin et al. 1991; McBean et al. 1971; Provost et al. 1993; Wilhelm et al. 1991). Most investigators have found a poor correlation between hair and plasma zinc levels since the zinc in hair does not exchange with the body zinc pool (McBean et al. 1971; Rivlin 1983). Furthermore, measurements of zinc in hair can be affected by extraneous contamination of hair, contamination by sweat, location of hair sample (distance from scalp), hair coloring, and rate of hair growth (McBean et al. 1971; Rivlin 1983). Although the nail is considered more resistant to washing procedures than hair, external contamination and uncertainties regarding the length and period of exposure reflected by the observed zinc concentration limit this measurement as a biomarker of exposure for zinc (Wilhelm et al. 1991).

2.5.2 Biomarkers Used to Characterize Effects Caused by Zinc

The respiratory tract is the most sensitive target organ for zinc following inhalation exposure. Inhalation of zinc oxide results in a syndrome referred to as metal fume fever. Symptoms include fevers, chills, cough, listlessness, and metallic taste. Although oxides of several heavy metals

(antimony, aluminum, arsenic, cadmium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, selenium, silver, and tin) and pyrolysis products of fluorocarbon polymers (polytetrafluoroethylene [Teflon] and fluorinated polyethylene propylene) also produce metal fume fever (Ellenhorn and Barceloux 1988), this group of symptoms may be used as a nonspecific biomarker to identify inhalation exposure to zinc oxide.

The target organs associated with oral zinc exposure include the gastrointestinal tract, blood, immune system, and pancreas. The toxic effects observed after oral exposure to zinc include nausea, vomiting, diarrhea, decreased hemoglobin and hematocrit levels, immune suppression, increased serum amylase and lipase, and decreased HDL cholesterol levels. (A more detailed discussion of effects associated with exposure to zinc is presented in Section 2.2.) However, nausea, vomiting, and diarrhea may be observed following exposure to any gastrointestinal irritant. Increases in serum amylase and lipase are also markers for pancreatic damage; therefore, any condition resulting in pancreatitis (i.e., biliary tract disease [gallstones], alcoholism, trauma, inflammation, blood-borne bacterial infections, viral infections, ischemia, and drugs such as azathioprine, thiazides, sulfonamides, and oral contraceptives) would result in similar increases in these enzymes (Cotran et al. 1989). A hypochromic microcytic anemia that is not responsive to iron supplements may indicate exposure to zinc; however, such anemia may also retlect copper, pyridoxine, or cobalt deficiency, lead intoxication, poor diet, or chronic blood loss (Suber 19S9).

Thus, none of the above-mentioned effects observed after exposure to zinc is specific to zinc exposure. However, the combination of these toxic effects may be indicative of zinc overexposure. Additional information on the health effects of zinc may be found in Section 2.2.2. Additional information on biomarkers for renal, hepatobiliary, immune, and nervous system effects may be found in the CDC/ATSDR (1990) and OTA (1990) reports listed in Chapter 8.

Increased erythrocyte metallothionein may be an index of zinc exposure in humans (Grider et’al. 1990). Daily supplementation of 50 mg zinc/day to subjects for at least 7 days caused a seven-fold increase in metallothionein concentration in erythrocytes. At least 3-4 days were required before an increase in metallothionein is observed. This biomarker of exposure is only useful for recent zinc exposure because the metallothionein levels decreased approximately a week after discontinuation of a 63-week supplementation of zinc (Grider et al. 1990). Fourteen days after discontinuation of zinc supplements, metallothionein levels were reduced by 61%.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Zinc is an essential element obtained from the diet. Many different metals and nutrients interact with the absorption, distribution, and excretion of zinc. However, information was not found concerning interactions that increase the toxicity of zinc or other substances in the presence of zinc. Zinc administration may increase the toxicity of lead; however, the data are conflicting (Cerklewski and Forbes 1976; Hsu et al. 1975).

Metallothionein, a sulfhydryl-rich protein inducible by certain divalent cations and a variety of other agonists, is involved in the interaction between zinc and other metals such as copper (Wapnir and Balkman 1991). Inhibition of intestinal copper absorption by zinc may demonstrate competition between the two metals at the brush border of the lumen (Wapnir and Balkman 1991). Dietary intake of copper (1, 6, and 36 mg/kg) or zinc (5, 30, and 180 mg/kg) do not significantly alter the absorption of the other (Oestreicher and Cousins 1988), but when zinc levels are much higher than copper levels, copper absorption is depressed (Fischer et al. 1981). High levels of dietary zinc are known to induce de novo synthesis of metallothionein in the intestinal mucosal cell. Both copper and zinc appear to bind to the same metallothionein protein; however, copper has a higher affinity for metallothionein than zinc and displaces the zinc that is attached to the metallothionein (Ogiso et al. 1979). Copper complexed with metallothionein is retained in the mucosal cell, relatively unavailable for transfer to plasma, and is excreted in the feces when the mucosal cells are sloughed off (Fischer et al. 1981; L'Abbe and Fischer 1984b). A number of factors influence the effect of dietary zinc on copper metabolism, including the amount of copper and zinc in the diet, the zinc-to-copper ratio, age of the individual, and the duration of exposure to high zinc levels (Johnson and Flagg 1986).

Physiological interactions of zinc and cadmium have been discussed in a number of reviews (EPA 1980c; NAS 1980; Underwood 1977). Exposure to cadmium may cause changes in the distribution of zinc, with accumulation of zinc in the liver and kidney. This accumulation in the liver and kidney may result in a deficiency in other organs, particularly if the dietary intake of zinc is marginal. *In vitro* data demonstrate that zinc and cadmium enter renal proximal cells by a saturable, carrier-mediated process and a non-saturable pathway (Gachot and Poujeol 1992). At low cadmium doses, cadmium and zinc compete for a common transport carrier system in renal proximal cells. It is hypothesized that, at high doses, the subcellular microtubule system is

disrupted by cadmium, which may interfere with changes in carrier configuration that are necessary for transport of the metals (modification of the cytoskeleton), and thereby lead to noncompetitive inhibition between cadmium and zinc (Gachot and Poujeol 1992). Combined treatment with cadmium and zinc in primary cultures of kidney cells resulted in enhanced toxicity of cadmium (Yoshida et al. 1993); however, pretreatment with a nontoxic concentration of zinc caused increased induction of metallothionein synthesis and partial protection against cadmium (Yoshida et al. 1993).

Cadmium is 10 times more efficient than zinc in metallothionein induction *in vitro* (Harford and Sarkar 1991). Induction by either cadmium or zinc alone is saturable; however, simultaneous administration of cadmium and zinc results in induction of metallothionein in an additive manner. The additive effect on metallothionein induction may involve binding of the metals either to two or more metallothionein promoter binding proteins or separate sites on the same promoter binding protein (Harford and Sarkar 1991).

Zinc acetate pretreatment in the mouse TRL-1215 cell line reduced single-strand DNA damage associated with cadmium exposure (Coogan et al. 1992). Diminished cadmium-induced DNA damage was not due to decreased cadmium burden in the zinc-pretreated cells. Instead, cadmium levels were actually greater than those in non-pretreated cells (Coogan et al. 1992). Metallothionein levels were elevated in these cells, suggesting that zinc pretreatment affects cadmium genotoxicity by inducing metallothionein which may sequester cadmium from genetic material. In contrast, simultaneous exposure to cadmium and zinc decreased cadmium accumulation in the cells, perhaps because of direct competition for a common transport mechanism (Coogan et al. 1992).

Zinc acetate reduced or prevented cadmium carcinogenesis in the prostate, in the testes, or at the injection site in rats (Gunn et al. 1963a, 1964; Waalkes et al. 1989). The effect of zinc on the cadmium-induced carcinogenesis appeared to be dependent on dose, route, and target site. Sustained levels of zinc inhibited cadmium-induced injection sarcomas but had no effect on the incidence of testicular Leydig cell tumors (Waalkes et al. 1989).

Excessive dietary zinc has been shown to induce a reversible copper deficiency and anemia in experimental animals (Magee and Matrone 1960; Murthy and Petering 1976; O'Dell 1969;

Underwood 1977; Wapnir and Balkman 1991). Similar effects have been seen in humans receiving long-term treatment with zinc (Porter et al. 1977; Prasad et al. 1978). However, no significant decreases in plasma copper levels were observed in humans receiving zinc for 6 weeks or 6 months (Henkin et al. 1976; Samman and Roberts 1987) or in mice administered zinc for l-12 weeks (Sutomo et al. 1992). A reduction in erythrocyte superoxide dismutase (an index of metabolically available copper), without a decrease in plasma copper levels, was exhibited following exposure to high amounts of ingested zinc (Fischer et al. 1984). These findings suggest that superoxide dismutase may be a sensitive indicator of zinc-copper interaction.

Cobalt has been demonstrated to induce seminiferous tubule damage and degeneration (vacuole formation, sloughing of cells, giant cell formation) in the testes of mice following exposure for 13 weeks (Anderson et al. 1993). Coadministration of cobalt and zinc chloride in the drinking water resulted in complete or partial protection in 90% of the animals. The sites of competitive interaction between zinc an cobalt were not established in the study; however, the authors postulated that the mechanism(s) may be similar to those involved in prevention of cadmium toxicity by zinc.

The effect of tin on heme biosynthesis appears to be dependent on the concentration of zinc (Chmielnicka et al. 1992). Oral administration of tin can affect the heme synthesis by inhibiting δ-aminolevulinic acid dehydratase (ALAD) activity in blood, Zinc is required for ALAD activity and provides a protective role in heme synthesis by increasing the activity of ALAD. It is postulated that when the tin and zinc are coadministered, these metals are probably attaching to similar binding sites in the ALAD enzyme (Chmielnicka et al. 1992).

Calcium decreases the bioavailability of zinc; the converse is also true (Heth and Hoekstra 1965; Spencer et al. 1992). Oral zinc administration is associated with decreased calcium levels in the serum and in the bone of rats (Yamaguchi et al. 1983). Zinc inhibited calcium uptake in rat brush border membrane vesicles, possibly by competing directly at high-affinity calcium binding sites (Roth-Bassell and Clydesdale 1991). The interaction of calcium and zinc is apparently dose related; intestinal absorption of calcium at a low calcium intake (230 mg/day) was inhibited at a high zinc intake of 140 mg/day but not at a lower zinc intake of 100 mg/day (Spencer et al. 1992).

Pretreatment with zinc has been shown to reduce hepatotoxicity induced by xenobiotics such as acetaminophen, bromobenzene, carbon tetrachloride, D-galactosamine, gentamicin, and salicylate (Cagen and Klaassen 1979; Gunther et al. 1991; Hu et al. 1992; Szymanska et al. 1991; Yang et al. 1991). The protective effect of zinc against carbon tetrachloride toxicity is dose dependent at high dose levels of zinc, probably because of sequestering of toxic metabolites of carbon tetrachloride by metallothionein (Cagen and Klaassen 1979). Similarly, the protective action of zinc against bromobenzene and acetaminophen appears to be associated with elevated metallothionein levels (Szymanska et al. 1991). Inhibition of lipid peroxidation may be the basis for the protective effect of zinc against hepatic damage induced by D-galactosamine in rats (Hu et al. 1992). Zinc may be elevating NADPH (nicotinamide adenine dinucleotide phosphate) content in the cell, resulting in regeneration of glutathione, which increases the antioxidative ability of hepatic cells. Salicylate-induced hepatic alterations (increased lipid droplets and iron, reduced glycogen) (Gunther et al. 1991) and gentamicin-induced proximal tubular necrosis (Yang et al. 1991) were diminished in rats pretreated with injections of zinc chloride and zinc sulfate, respectively. This finding corresponded to a dramatic increase in metallothionein content with combined treatment of salicylate and zinc compared to a less significant increase with salicylate alone.

Animal studies suggest that the administration of zinc may also inhibit tumor growth. Forty weeks after exposure, the incidence of injection site sarcomas was 40-60% in rats receiving simultaneous intramuscular administration of nickel subsulfide and zinc oxide compared to an incidence of 100% following administration of nickel subsulfide alone (Kasprzak et al. 1988). Supplementing drinking water with zinc sulfate reduced the incidence of 9,10-dimethyl-1,2-benzanthraceneinduced tumors in the cheek pouches of mice (Poswillo and Cohen 1971). Zinc decreased DNA synthesis in hepatomas induced by 3’-methyl-4-dimethylaminoazobenzene (Duncan and Dreosti 1975). The investigators speculated that the changes were due to inhibited cell division cycle at the level of DNA replication.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to zinc than will most persons exposed to the same level of zinc in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase

susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects on clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

No specific data regarding human subpopulations that are unusually susceptible to the toxic effects of zinc were located. Healthy elderly people have been shown to have greater daily zinc intake than housebound elderly people (Bunker et al. 1987; Prasad 1988). Data from animal studies indicate that certain human subpopulations may be more susceptible to excess zinc because of zinc's depleting effect on copper (Underwood 1977). People who are malnourished or have a marginal copper status may be more susceptible to the effects of excessive zinc than people who are adequately nourished (Underwood 1977).

Hepatic zinc levels are elevated in patients with hemochromatosis, a genetic disease associated with increased iron absorption from the intestine (Adams et al. 1991). The chronic iron loading that occurs could result in hepatic metallothionein induction leading to the accumulation of zinc because metallothionein has a greater affinity for zinc than iron. These individuals may, therefore, have a greater likelihood of developing toxicity with zinc exposure levels that do not normally result in any symptoms in the general population.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to zinc. However, because some of the treatment discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to zinc. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

General recommendations for the management and treatment of patients following acute exposure to zinc include removal of the victim from the contaminated area and removal and isolation of contaminated clothing, jewelry, and shoes (Bronstein and Currance 19818; Stutz and Janusz 1988). Excess contaminant is gently brushed away and excess liquids blotted with absorbent material. Measures that are appropriate to the route of exposure are taken to remove zinc from the body. Exposed eyes are flushed immediately with water, followed as soon as possible with irrigation of each eye with normal saline. Exposed skin is washed immediately with soapy water. Administration of ipecac to induce emesis, gastric lavage, ingestion of activated charcoal, and cathartics have been recommended to decrease the gastrointestinal absorption of zinc (Burkhart et al. 1990; Ellenhorn and Barceloux 19%). Because zinc causes nausea and vomiting following exposure by the oral route, use of emetic agents may be unnecessary. Ipecac administration may be contraindicated following ingestion of caustic zinc compounds such as zinc chloride. The large amounts of phosphorus and calcium in milk and cheese, and phytate in brown bread, may reduce absorption of zinc from the gastrointestinal tract (Pecoud et al. 1975). Therefore, if vomiting and diarrhea are not prohibitive, ingestion of dairy products or brown bread may also reduce gastrointestinal absorption of zinc. In a study of intestinal absorption of zinc in iron-deficient mice, the uptake of zinc from the gut was inhibited by adding iron to the duodenal loop system. The proposed mechanism was that iron and zinc shared a common gut mucosal binding site (Hamilton et al. 1978). However, it is unknown whether ingestion of iron supplements would be effective in reducing absorption of zinc overdoses.

2.8.2 Reducing Body Burden

Zinc is an essential trace element that is normally found in tissues and fluids throughout the body and is under homeostatic control (NAS/NRC 1989b). Increased levels have been observed in the heart, spleen, kidneys, liver, bone, and blood of animals following subchronic oral exposure to zinc (Llobet et al. 1988a) indicating that some zinc accumulation occurs during excess intakes. The greatest increases were observed in bone and blood.

Administration of the chelating agent, calcium disodium ethylene diaminetetraacetate ($CaNa_2EDTA$), is the treatment of choice for reducing the body burden of zinc in humans

following exposure to high levels (Ellenhorn and Barceloux 19%). Ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and dimercaprol (BAL) are the most common antidotes used in the treatment of human zinc intoxications (Murphy 1970; Spencer and Rosoff 1966). Markedly elevated serum zinc levels in a young child who ingested a zinc chloride solution were normalized by intravenously administering a single small dose of $CaNa_2EDTA$ (11.5 mg/kg) (Potter 1981). Use of chelation therapy (administration of BAL) was reported in a case study of a 16-year-old boy who ingested 12 g of metallic zinc (Murphy 1970). The boy exhibited lethargy and elevated blood zinc levels that were both reversed following intramuscular administration of BAL. Chelation therapy has been demonstrated to increase the urinary excretion of zinc 22-fold (Spencer and Rosoff 1966). Intravenous and nebulized N-acetylcysteine (another metal chelating agent) have also been observed to increase urinary zinc excretion and decrease plasma levels following inhalation of zinc chloride smoke (Hjortso et al. 1988).

The efficacy of 16 different chelating agents as possible antidotes for acute oral zinc exposure has been determined in mice (Llobet et al. 1988b). The most efficient chelators were DTPA, cyclohexanediamine-tetraacetic acid (CDTA), and EDTA. Increased urinary levels of zinc and decreased bone and liver zinc levels were observed following administration of the chelators. The maximum efficiency of the chelators was observed when they were administered 0-.167-12 hours after zinc exposure (Domingo et al. 1988a, 1988b).

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

Anemia has been observed in humans and animals after oral exposure to zinc. It has been postulated that excess zinc intake may result in copper deficiency (mechanisms of action are discussed in Section 23.5). The anemia observed following zinc intake is believed to be caused by the copper deficiency. Administration of copper has been shown to be effective in increasing the hemoglobin levels (Porter et al. 1977; Smith and Larson 1946).

The exact mechanism of metal fume fever (a syndrome consisting of a leukocytosis with chills, fever, cough, myalgias, headache, weakness, and dyspnea) is unknown (Ellenhorn and Barceloux 1988), but respiratory tract inflammation and the development of an immune complex reaction have been proposed (McCord 1960).

In severe cases, inhalation of zinc chloride has resulted in advanced pulmonary fibrosis and fatal respiratory distress syndrome (Evans 1945; Hjortso et al. 1988; Milliken et al. 1963). L-3,4-Dehydroproline was given to two soldiers after inhaling a high concentration of zinc chloride smoke (also contained other chemicals) in an attempt to inhibit collagen deposition in the lungs (Hjortso et al. 1988). This therapy did not prevent respiratory failure.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of zinc is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of zinc.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of Zinc

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to zinc are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of zinc. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs.” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989) is

ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

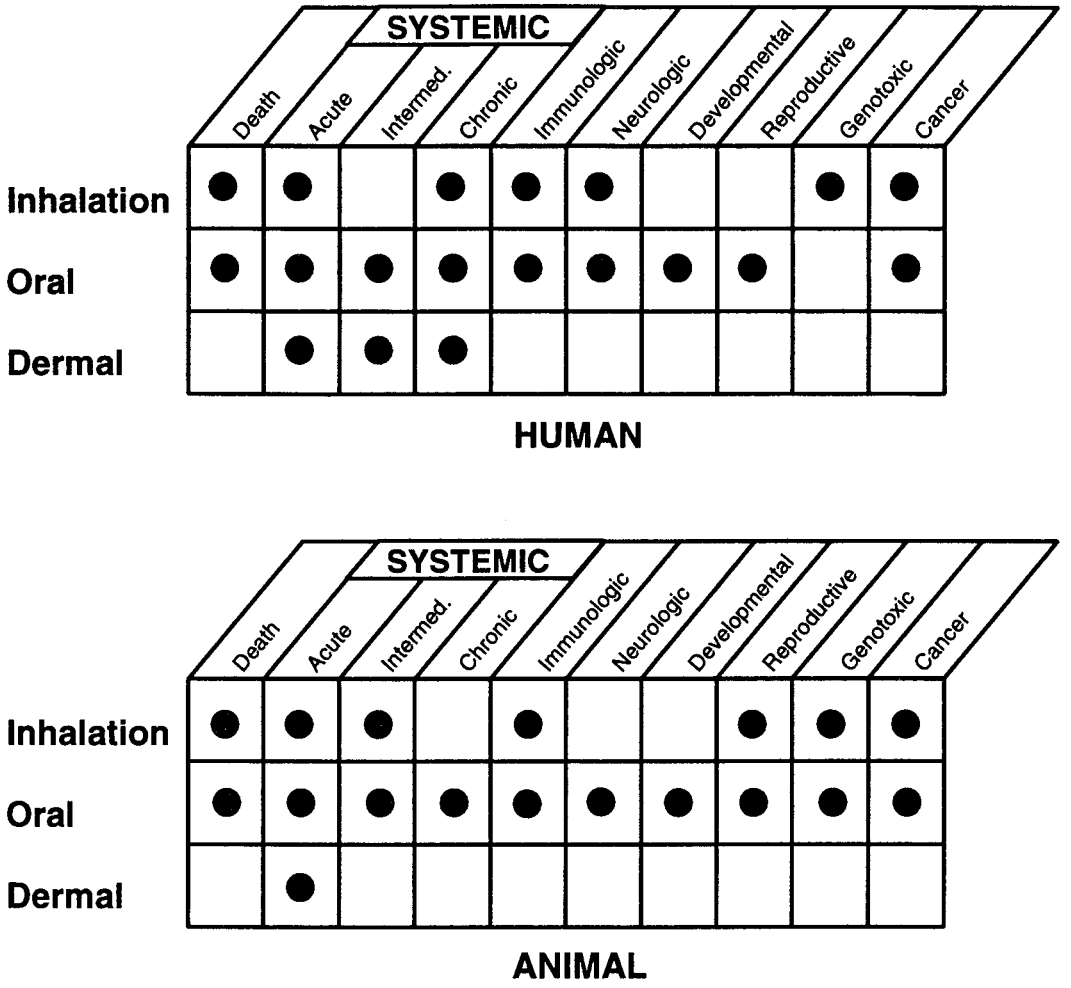
Figure 2-3 indicates whether a particular health effect end point has been studied for a specific route and duration of exposure. There is little information concerning death in humans after inhalation, oral, or dermal exposure to zinc. Several case studies report death after exposure to extremely high levels of zinc chloride and other components of zinc chloride smoke (Evans 1945; Hjortso et al. 1988; Milliken et al. 1963).

Systemic effects of acute inhalation exposure to generally unspecified levels of various zinc compounds in humans have been reported in several clinical case studies (Blanc et al. 1991; Brown 1988; Hjortso et al. 1988; Matarese and Matthews 1966; Vogelmeier et al. 1987). Case studies and experimental studies of systemic effects in humans following acute, intermediate, and chronic oral exposures are available (Anonymous 1983; Black et al. 1988; Brandao-Neto et al. 1990a; Chandra 1984; Chobanian 1981; Hale et al. 1988; Hallbook and Lanner 1972; Hoffman et al. 1988; Hooper et al. 1980; Malo et al. 1990; Moore 1978; Patterson et al. 1985; Porter et al. 1977; Potter 1981; Prasad et al. 1978). Experimental studies in humans following acute, intermediate, and chronic dermal exposures were located for hematological, dermal, and ocular effects (Agren 1990; Evans 1945; Fischer et al. 1984; Turner 1921; Yadrick et al. 1989).

Information concerning respiratory effects of acute inhalation exposure to zinc in animals is available (Amdur et al. 1982; Drinker and Drinker 1928; Lam et al. 1982, 1988). One study (Marrs et al. 1988) was located regarding other systemic effects in animals following inhalation exposure to zinc for an intermediate exposure duration. Information regarding systemic effects of zinc following oral exposure in animals is available for acute, intermediate, and chronic exposure durations (Allen et al. 1983; Anderson and Danylchuk 1979; Aughey et al. 1977; Bentley and Grubb 1991; Domingo et al. 1988a; Drinker et al. 1927; Jenkins and Hidiroglou 1991; Katya-Katya et al. 1984; Klevay and Hyg 1973; Llobet et al. 198Sa; Maita et al. 1981; Straube et al. 1980; Walters and Roe 1965). One acute dermal study evaluated dermal irritancy in animals (Lansdown 1991).

Immunological effects were reported in humans following inhalation exposure to zinc oxide (Blanc et al. 1991; Farrell 1987). Another study reported potential adverse immunological effects

FIGURE 2-3. Existing Information on Health Effects of Zinc



following oral exposure of humans (Chandra 1984). Clinical symptoms suggestive of neurological effects have been reported by humans following inhalation exposure (Rohrs 1957; Sturgis et al. 1927; Wilde 1975) or oral exposure (Anonymous 1983; Murphy 1970; Potter 1981) to zinc. There were studies that examined reproductive and developmental effects in women orally exposed to zinc during their pregnancies (Kynast and Saling 1986; Mahomed et al. 1989; Simmer et al. 1991).

One study examined immunological and reproductive effects in animals following inhalation exposure to zinc chloride (Marrs et al. 1988). Immunological and neurological end points were evaluated in animals following oral exposure to zinc (Bleavins et al. 1983; Kozik et al. 1980, 1981; Schiffer et al. 1991). Information regarding developmental and reproductive effects in animals after oral exposure to zinc is available (Cox et al. 1969; Ketcheson et al. 1969; Kinnamon 1963; Mulhern et al. 1986; Pal and Pal 1987; Schlicker and Cox 1968; Sutton and Nelson 1937). Studies regarding genotoxicity in animals after inhalation and oral exposures to zinc are limited (Gupta et al. 1991; Kowalska-Wochna et al. 1988; Voroshilin et al. 1978).

Epidemiological studies regarding carcinogenicity after inhalation and oral exposure to zinc are available (Logue et al. 1982; Neuberger and Hollowell 1982; Philipp et al. 1982; Stocks and Davies 1964); however, they were not well controlled and the data are of little significance. Studies are available regarding carcinogenicity in animals after inhalation and oral exposure to zinc (Marrs et al. 1988; Walters and Roe 1965). However, the studies have several deficiencies that limit their usefulness.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Symptoms of metal fume fever (headache, fever, leukocytosis, myalgias) have been observed in humans acutely exposed to airborne zinc oxide (Blanc et al. 1991; Brown 1988; Drinker et al. 1927b; Sturgis et al. 1927). Acute oral exposure to zinc has resulted in gastrointestinal disturbances (abdominal pain, nausea, vomiting, esophageal erosion), evidence of pancreatic damage (increased serum amylase and lipase levels), and decreased levels of serum cortisol in humans (Anonymous 1983; Brandao-Neto et al. 1990a; Chobanian 1981; Murphy 1970; Potter 1981). Acute dermal exposure to zinc oxide has not been shown to be irritating to human skin (Agren 1990). Toxic effects similar to those observed for metal fume fever have been observed in guinea pigs (Amdur et al. 1982; Lam et al. 1985). In addition to

LD_{50} data, only one reliable study assessed the acute oral toxicity of zinc compounds in animals. Pancreatic, gastrointestinal, and liver damage were observed in sheep (Allen et al. 1983). It is doubtful that sheep (ruminant animals) are an appropriate model for toxicity of orally administered zinc in humans. The dermal toxicity of several zinc compounds has been tested in rabbits, guinea pigs, and mice (Lansdown 1991). Zinc acetate, zinc chloride, and zinc sulfate have irritating properties. Skin irritation was not observed in rabbits, guinea pigs, or mice after zinc oxide paste application (Lansdown 1991).

The animal data (Amdur et al. 1982; Drinker and Drinker 1928; Lam et al. 1982, 1988) corroborate occupational exposure studies that indicate metal fume fever is an end point of concern. However, other possible targets of toxicity have not been examined. Thus, an acute inhalation MRL cannot be derived. A large amount of the human oral exposure data is in the form of case reports, and a great deal of uncertainty exists regarding the dose levels. The uncertainty about whether sheep are a good model for humans precludes using these data to derive an oral MRL for acute-duration exposure. Additional studies involving acute exposure to zinc compounds by all routes of exposure would be helpful to identify target organ and doseresponse relationships. There are groups who may be exposed to zinc at hazardous waste sites for brief periods; therefore, this information is important.

Intermediate-Duration Exposure. Metal fume fever was observed in an individual exposed to zinc fumes and zinc powder for approximately 1 month (Malo et al. 1990). Anemia and decreased levels of HDL cholesterol have been observed in humans taking high doses of zinc supplements (Chandra 1984; Hoffman et al. 1988; Hooper et al. 1980). Intermediate-duration dermal exposure to zinc oxide dust has resulted in pustuIar lesions, but these lesions were attributed to clogging of the sebaceous glands resulting from poor hygiene (Turner 1921). Rats, mice, and guinea pigs exposed to smoke containing zinc chloride and other compounds had evidence of lung irritation (Marrs et al. 1988). No intermediate-duration animal dermal studies were located. In animals that ingested zinc for an intermediate duration, anemia and kidney and pancreas damage were observed (Bentley and Grubb 1991; Drinker et al. 1927d; Jenkins and Hidiroglou 1991; Llobet et al. 1988a; Maita et al. 1981; Straube et al. 1980).

Only one case report regarding human intermediate-duration inhalation exposure was located, and this study did not report the exposure level (Malo et al. 1990). Thus, an intermediate-duration

inhalation MRL could not be derived. The less serious LOAELs (decreased serum HDL cholesterol) identified in the Hooper et al. (1980) and Chandra (1984) human oral exposure studies could be used as the basis of an intermediate-duration oral MRL; however, evidence regarding this effect is inconsistent (Bogden et al. 1988; Hale et al. 1988; Samman and Roberts 1988). In addition, the resulting MRL would be ≈l0-fold lower than the RDA of 15 mg/day (0.21 mg zinc/kg/day). An intermediate-duration oral MRL was derived for zinc based on hematological effects (decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase) in women given zinc gluconate supplements for 10 weeks (Yadrick et al. 1989). The toxic effects of intermediate-duration exposure to zinc compounds are relatively well characterized for the oral route. There are insufficient toxicokinetic data to determine if the toxic effects observed following oral exposure would occur following inhalation or dermal exposure. Inhalation and dermal studies would be useful to determine possible target organs and dose-response relationships. There are populations surrounding hazardous waste sites that might be exposed to zinc compounds for similar durations.

Chronic-Duration Exposure and Cancer. No exposure-related effects on lung function were observed in a group of welders chronically exposed to zinc (Marquart et al. 1989). Anemia has been observed in humans following ingestion of high doses of zinc supplements (Broun et al. 1990; Hale et al. 1988; Porter et al. 1977; Prasad et al. 1978). Chronic-duration dermal exposure to zinc oxide dust has resulted in pustular lesions, but these were attributed to clogging of the sebaceous glands resulting from poor hygiene (Batchelor et al. 1926). No chronic-duration inhalation or dermal studies in animals were located. Pancreatic damage was observed in mice after chronic exposure to zinc sulfate in drinking water (Aughey et al. 1977).

A chronic-duration inhalation MRL could not be derived for zinc because neither of the inhalation studies reported the levels of airborne zinc. Due to a lack of adequate chronicduration oral studies, the intermediate-duration oral MRL was adopted as the chronic-duration oral MRL, based on hematological effects (decreased hematocrit, serum ferritin, and erythrocyte dismutase) in women given zinc gluconate supplements for 10 weeks (Yadrick et al. 1989). Additional studies involving chronic exposure to zinc compounds by all routes of exposure would be helpful to identify dose-response relationships.

Although there are several human and animal carcinogenicity studies, the limitations of these studies preclude their use in assessing the carcinogenicity of zinc (Logue et al. 1982; Neuberger and Hollowell 1982; Walters and Roe 1965). Carcinogenicity studies by all routes of exposure would be useful.

Genotoxicity. Several *in vitro* microbial gene mutation assays were negative (Marzin and Vo Phi 1985; Nishioka 1975; Thompson et al. 1989; Venitt and Levy 1974; Wong 19&S), but evidence from gene mutation assays in mammalian cells is mixed (Amacher and Paillet 1980; Thompson et al. 1989). An increase in the occurrence of chromosomal aberrations was observed *in vitro* in human lymphocytes (Deknudt and Deminatti 1978) and *in vivo* in rats and mice (Deknudt and Gerber 1979; Gupta et al. 1991; Kowalska-Wochna et al. 1988; Voroshilin et al. 1978). Increased sister chromatid exchange was observed *in vivo* in rat bone marrow (Kowalska-Wochna et al. 1988). However, while there are sufficient *in vivo* data establishing the clastogenicity of zinc, data regarding the mutagenicity of zinc are conflicting. Studies designed to assay different types of genotoxicity (i.e., mutagenicity in mammalian cells, effect of excess zinc on DNA replication) would be useful for determ.ining the genotoxic potential of zinc.

Reproductive Toxicity. No complications occurred in the pregnancies of women exposed to daily doses of zinc sulfide during the last two trimesters (Mahomed et al. 1989). No studies were located regarding the reproductive toxicity of zinc in humans after inhalation or dermal exposure. Increased pre-implantation loss and reproductive dysfunction in rats were observed in oral exposure studies (Pal and Pal 1987; Sutton and Nelson 1937). No histological changes in reproductive organs were observed in rats, mice, or guinea pigs following inhalation exposure to zinc chloride smoke, but reproductive function was not assessed (Marrs et al. I988). No dermal reproductive toxicity studies in animals were located. Inhalation and dermal studies assessing reproductive function would be useful to determine whether zinc has the potential to cause reproductive effects by these routes. An oral reproductive toxicity study in a different animal strain as well as a multigeneration study, including reproductive organ pathology, would be useful

Developmental Toxicity. No studies were located regarding the potential of zinc to cause developmental effects in humans after inhalation or dermal exposure. In a very brief report of a human study in which pregnant women received high-doses of zinc supplements during the last

trimester of pregnancy, an increased incidence of stillbirths and one premature delivery were observed (Kumar 1976). This study, however, has many limitations. Increased fetal resorptions were observed in rats after oral exposure to zinc (Schlicker and Cox 1968). No studies were located regarding developmental toxicity in animals after inhalation or dermal exposure to zinc. Additional inhalation, oral, and dermal exposure studies in animals would be useful to predict whether developmental effects should be a concern for humans exposed to zinc.

Immunotoxicity. Metal fume fever is believed to be an immune response to zinc oxide. A correlation between the concentration of airborne zinc and the number of all types of T cells (helper, inducer, suppressor, and killer) in the bronchoalveolar lavage fluid of humans, possibly related to the onset of metal fume fever, was observed in an acute-duration inhalation study (Blanc et al. 1991). Impaired immune response in humans has been reported in an intermediate-duration oral study (Chandra 1984). No immune effects were observed in mice after oral exposure to zinc (Schiffer et al. 1991). There is some limited information to suggest that the immune system is a target of zinc toxicity. A battery of immune function tests after inhalation, oral, and dermal exposure to zinc compounds would be useful in determining if zinc is immunotoxic.

Neurotoxicity. Staggering gait and hallucinations were reported in an individual who intentionally inhaled metallic paint aerosols (Wilde 1975). Because there was simultaneous exposure to copper and hydrocarbons, this study cannot be used to assess the neurotoxic potential of zinc. Nonspecific signs and symptoms of neurotoxicity (light-headedness, dizziness, headache, lethargy) have been reported by humans following acute oral exposure to zinc (Murphy 1970; Potter 1981). Very limited data suggest that high oral doses of zinc can cause minor neuron degeneration and alteration of secretion of the hypothalamus in rats (Kozik et al. 1980, 1981). No studies were located regarding neurotoxic effects in animals after inhalation or dermal exposure to zinc. Additional studies by all routes of exposure would be useful to determine if exposure to zinc compounds would result in neurotoxicity.

Epidemiological and Human Dosimetry Studies. Acute high-level exposure to zinc by inhalation resulted in respiratory irritation and metal fume fever (Blanc et al. 1991; Hjortso et al. 1988; Johnson and Stonehill 1961; Linn et al. 1981; Schenker et al. 1981; Sturgis et al. 1927). Welders are a subpopulation of workers who have a high potential for exposure to zinc oxide.

Most of the available studies did not report exposure levels or used a small number of subjects. Studies that correlate occupational exposure to zinc with health effects would be useful. A number of human oral exposure studies have shown that excess levels of zinc can result in anemia, pancreatic damage, decreased serum HDL cholesterol levels, and immunotoxicity (Black et al. 1988; Chandra 1984; Hooper et al. 1980). There are insufficient data for establishing dose-response relationships. Studies designed to establish dose-response relationships would be useful for establishing cause/effect relationships and future monitoring of individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect

Exposure. Increased serum and urine levels of zinc were observed in humans and animals after inhalation, oral, or dermal exposure to zinc (Bentley and Grubb 1991; Brandao-Neto et al. 1990b; Hallmans 1977; Keen and Hurley 1977). However, the relationships between zinc exposure levels and the levels of zinc in biological fluids have not been established. Hair and nail samples may be a potential biomarker for long-term zinc exposure (McBean et al. 1971; Rivlin 1983; Wilhelm et al. 1991); however, no correlation has been demonstrated between these parameters and zinc exposure levels. Development of a biomarker with more exposure and dose data would aid in future medical surveillance that could lead to better detection of zinc exposure.

Effect. Several potential biomarkers for the effects of zinc have been identified. These include increased levels of serum amylases and lipase, indicative of pancreatic damage; non-iron responsive anemia; and decreased HDL cholesterol levels (Suber 1989). However, these biomarkers of effect are not specific for zinc. These biomarkers cannot be used for dosimetry. A potential biomarker of exposure for recent exposures to zinc is increased erythrocyte metallothionein concentrations (Grider et al. 1990). Further investigation of serum biomarkers of effect, particularly for chronic exposure, in zinc-exposed populations would be useful to determine whether exposed populations may be experiencing adverse health effects as the result of zinc exposures.

Absorption, Distribution, Metabolism, and Excretion. Absorption of zinc in humans after oral exposure to high levels has been well described (Aamodt et al. 1983; Hunt et al. 1991; Reinhold et al. 1991; Sandstrom and Abrahamson 1989; Sandstrom and Cederblad 1980; Sandstrom and

Sandberg 1992; Spencer et al. 1985). However, quantitative evidence of zinc absorption in humans after inhalation or dermal exposure is very limited. It is known that workers exposed to zinc oxide fumes who experience toxic effects have elevated levels of zinc in plasma and urine (Hamdi 1969). However, it remains to be established whether the elevated levels are the result of the pulmonary absorption or of the swallowing of particles leading to gastrointestinal absorption. Toxic effects have also been observed in humans after dermal exposure (DuBray 1937) indicating dermal absorption.

Information regarding the absorption of zinc in animals following inhalation exposure was limited to lung retention data (Gordon et al. 1992; Hirano et al. 1989). However, there was information to assess the extent of absorption following oral exposure (Davies 1980; Galvez-Morros et al. 1992; Johnson et al. 1988; Weigand and Kirchgessner 1992). Evidence is limited regarding dermal absorption in animals, but it indicates that zinc sulfate and zinc oxide can penetrate the skin (Agren 1990; Agren et al. 1991; Gordon et al. 1981; Hallmans 1977). Mechanistic data on the oral absorption is reported by Hempe and Cousins (1992); however, there is a lack of information regarding the mechanism of action of inhalation and dermal exposures.

Information on physiological levels and zinc distribution following subtoxic short-term exposures to zinc in humans and animals is abundant (NAS/NRC 1979; Wastney et al. 1986). Blood levels of zinc have been determined in humans following oral exposure to zinc sulfate (Neve et al. 1991; Statter et al. 1988; Sturniolo et al. 1991). Increased zinc tissue content has been seen after shortterm oral exposure in humans (Cooke et al. 1990; He et al. 1991; Llobet et al. 198Sa; Schiffer et al. 1991; Weigand and Kirchgessner 1992). Studies on tissue distribution in humans following high exposure to zinc for inhalation, oral, and dermal would be useful. There were no studies regarding blood or tissue distribution after acute, high-level exposures to zinc in animals following inhalation or dermal exposure. Additional mechanistic data on the transfer of zinc from respiratory and dermal absorption sites to the blood would be useful.

The principal excretion route of ingested zinc is through the intestines (Davies and Nightingale 1975; Reinhold et al. 1991; Wastney et al. 1986). There is a lack of information regarding the excretion of zinc in both animals and humans following inhalation and dermal exposure.

Therefore, additional studies designed to assess the toxicokinetic properties of zinc following inhalation and dermal exposures would be useful.

Comparative Toxicokinetics. Data suggest that humans and animals have similar target organs of zinc toxicity (Allen et al. 1983; Aughey et al. 1977; Black et al. 1988; Blanc et al. 1991; Brown 1988; Chandra 1984; Chobanian 1981; Drinker et al. 1927b, 1927d; Hoffman et al. 1988; Hooper et al. 1980; Katya-Katya et al. 1984; Kievay and Hyg 1973; Lam et al. 1982, 1985, 1988; Maita et al. 1981; Moore 1978; Murphy 1970; Smith and Larson 1946; Straube et al. 1980; Sturgis et al. 1927). Toxicokinetic studies have been performed in both humans and animals following oral exposure; however, data are limited for inhalation and dermal exposures. The animal model used most often to evaluate the toxicokinetics of zinc are rats (Agren et al. 1991; Alexander et al. 1981; Galvez-Morros et al. 1992; Hirano et al. 1989; Llobet et al. 1988a; Weigand and Kirchgessner 1992) and may be a good model for assessing the kinetics of zinc in humans.

Methods for Reducing Toxic Effects. No established methods or treatments for reducing the absorption of zinc were located. Studies that examined the effectiveness of emetics and cathartics in the prevention of zinc absorption would be useful. Once absorbed from the gastrointestinal tract, zinc bound to plasma albumin is distributed to the rest of the body. Zinc has a high affinity for proteins, and a number of chelating agents are effective in increasing urinary excretion of zinc following acute- and intermediate-duration administrations (Domingo et al. 1988a, 1988b; Llobet et al. 1989). Studies designed to examine the effectiveness of chelating agents following chronic zinc exposure would be useful in determining treatments to reduce the zinc body burden. Very little information is known about the absorption and distribution of zinc following inhalation or dermal exposure. Studies to determine the mechanisms of absorption and distribution would be useful for developing treatments or methods for reducing the toxic effects of zinc after inhalation or dermal exposure.

Although the exact mechanisms of many of the toxic actions of zinc are not known, the pathogenesis of metal fume fever following inhalation exposure (McCord 1960; Mueller and Seger 1985) and anemia following oral exposure (Prasad et al. 1978) are known. Studies to more clearly elucidate the mechanisms involved in metal fume fever and anemia and to determine the mechanisms involved in pancreatic damage and decreased HDL cholesterol levels would be useful. Therapy for metal fume fever is mainly supportive (Mueller and Seger 1985). Administration of

copper has been shown to be effective in alleviating zinc-induced anemia (Porter et al. 1977). Research into methods useful for mitigating metal fume fever and other adverse effects of zinc would be helpful.

2.9.3 On-going Studies

Currently, D.W. Christianson (University of Pennsylvania, Philadelphia, Pennsylvania) and C.A. Fierke (Duke University, Durham, North Carolina) are looking at redesigning the zinc binding site of the human carbonic anhydrase II enzyme and E. Kimura's group (Hiroshima University, Hiroshima, Japan) is examining the roles of zinc(I1) ion in zinc enzymes.